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Invited Review

Comments on Report of a Joint FAO/WHO Expert Consultation on Protein Quality Evaluation, Rome 1990

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A joint (FAO/WHO) expert consultation on protein quality evaluation was held in Bethesda, Maryland, USA, December 4–8, 1989. A report from this meeting is now available from the FAO of the United Nations, Rome (1990).

Based on results of collaborative studies undertaken in 1987 and 1988, and on recent improvements in amino acid methodology, the Codex Committee on Vegetable Proteins (CCVP) at its fifth session in Ottawa, Canada, in February 1989, endorsed the use of the 1985 FAO/WHO/UNU suggested pattern of amino acid requirements of a 2–5-year-old child as the reference for calculating amino acid scores, and agreed that amino acid scores (based on the amount of the single most limiting amino acid) corrected for true digestibility of protein (as determined by the rat balance method) is the most suitable routine method for assessing the protein quality of most vegetable protein products and other food products. Because the methodology used to measure protein quality had broad implications beyond its purview, the CCVP recognized the need for the wider scientific community to address issues such as human requirements for essential amino acids, amino acid methodology, protein digestibility, and amino acid availability. The committee accordingly recommended that a joint FAO/WHO expert consultation should be held in order to

Abbreviations: PER = protein efficiency ratio; USDA = United States Department of Agriculture; TD = True digestibility.

review the issues. The participants were requested to review the results of studies carried out by the ad hoc Working Group on Protein Quality Measurement and to evaluate the protein digestibility-corrected amino acid score method for its usefulness in human nutrition.
The objectives of the meeting were:
- to review present knowledge of protein quality evaluation;
- to discuss various techniques used in evaluating protein quality; and
- to *specifically* evaluate the method recommended by the CCVP held in Ottawa, 1980, i.e., amino acid score corrected for digestibility.

The meeting was a direct follow-up to a recommendation by the CCVP at its fifth session held in Ottawa in February, 1989. It was stressed to the experts the importance of vegetable proteins in the diet of both developed and developing countries, and in international trade, and therefore, the need to develop adequate methodologies for the assessment of their nutritive quality. It was hoped that developing world-wide standards and guidelines would help eliminate non-tariff barriers and facilitate international trade of vegetable protein products.

For some time the use of an amino-acid score has been advocated as an alternative to the PER. Although, clearly, the quality of some proteins can be assessed directly by using amino-acid-score values, others cannot because of poor digestibility and/or bioavailability. Consequently, both amino acid composition and digestibility measurements are considered necessary to accurately predict the protein quality of foods for human diets. The measurement of protein quality can have a broad economic impact on foods, food ingredients, and national food policy. Protein quality measurement should evaluate the protein relative to human requirements. Since protein value is related primarily to the amino acid content relative to human amino acid needs, the primary criterion for judging any food protein should be its essential amino acid content relative to human amino acid requirements. Methods of measuring protein quality which correlate with human requirements will have a favorable economic impact on food cost and availability. The greater the deviation of the method from accurately reflecting the amino acid requirement, the greater the cost will be to consumers, food producers, and governments.

The methods presently used for measuring protein value of foods were established, when information was not available on human amino acid requirements. Since most of these methods use a rat assay, they are in large part related to the amino acid requirements of the rat rather than the human. This is particularly misleading since the rat appears to have a much higher requirement for sulphur amino acids than the human does. The rat also has a higher requirement for histidine, isoleucine, threonine, and valine.

The rat growth assay method employs casein as the reference protein. However, rat growth is influenced by both the amino acid content of the casein and the amino acids required by the rat. Thus, due to the high sulphur amino acid requirement of the rat, the assay is primarily a measure of the sulphur amino acid content of casein.

The casein/rat growth assay procedures do not accurately judge a food for human diets. This inaccuracy in the assessment of protein value can result in major errors in national policy and selection of food and in

economic loss to consumers and producers. This can be avoided by directly comparing food proteins to human amino acid pattern.

There are numerous restrictive national policies based on meeting a specific PER value which, in developed cuntries, has resulted in increased costs of foods to the general population with no perceivable benefit. The developing countries where food supplies are limited and funds are limited for purchasing foods for the undernourished, this unnecessary dependence on rat growth assay for the selection of food imported or purchased for social programs may have vital significance.

The use of amino-acid scores related to human requirements would provide a realistic basis for defining the value of food proteins based on human needs rather than the needs of the growing rat. It would give the food processor the opportunity to formulate more nutritious foods with a better and more economical food selection.

The validity of early studies were limited by lack of standardized and reproducible procedures for determining tryptophan and sulphur amino acids, by insufficient data on digestibility of protein and bioavailability of amino acids in foods, and by uncertainty about human amino acid requirements to be used for the scoring pattern. During the last few years, significant advancements have been made in standardizing amino acid methodology, in reaching a consensus about human amino acid requirements, and in obtaining information about digestibility of protein and bioavailability of amino acids in a number of protein sources. These developments have facilitated the use of an amino-acid-scoring procedure adjusted for digestibility, which is a better predictor of protein quality for humans than rat growth methods and is, in most cases, the only practical approach. The consultation participants reviewed principal methods of analysis of amino acids and the following conclusions and recommendations were made:

1) Modern amino-acid analysis can provide data with a repeatability within laboratory of about 5 %, and a reproducibility between laboratories of about 10 %. It is recommended that this variability be considered acceptable for the purposes of calculating amino-acid score. To achieve such results requires careful attention to many aspects of the protocols, including replicating the complete analytical procedure.
2) It is recommended that further studies be undertaken to standardize the hydrolytic and oxidation procedures and improve accuracy of the procedures to further reduce interlaboratory variation.
3) It is recommended that collaborative trials be undertaken of the new HPLC methods.
4) Amino acids data should be reported as mg amino acid/g N or be converted to mg amino acid/g protein by use of the factor 6.25. *No other food specific protein factor should be used.*
5) FAO should update their publication *Amino acid content of foods and biological data on proteins* and commission new analyses of foods where there are insufficient reliable data.
6) Reliable national tables of amino acid composition of products which have been clearly defined in terms of composition and processing should be developed.

Recognizing the need for amino acid scoring patterns which can be used to assess quality of food protein sources and diets in all age groups the consultation participants decided that the scoring pattern proposed for the preschool child, which is based on various criteria of amino acid adequacy, is robust and represents the best available estimates of IAA (indispensable amino acids) requirements for this age group.

In the absence of sufficient new experimental data to determine more definitively a scoring pattern for older children and adults, it was agreed that, in the interim, the preschool child scoring pattern should be employed for all ages, except for infants.

It was recognized, however, that the use of this pre-school amino acid scoring pattern means that there will be some uncertainty about the extent to which protein quality will be accurately predicted for older children and adults, and that there may be some chance of the overestimation of protein needs and underestimation of protein quality. However, the consultation participants considered that, in this event, this would result in a smaller error when protein quality is evaluated, than when the current FAO/WHO/UNU scoring pattern for adults is used.

The consultation participants therefore recognized the urgent need for further research in older children and adults to supplement the existing information and ultimately define the needs for IAA in these age groups. This should include research to identify functional indicators of amino acid adequacy.

They also recognized the need and importance to confirm and reinforce the existing information on IAA requirements for infants and preschool-aged children, since they form the basis of this panel's recommendation for an amino-acid-scoring pattern to evaluate protein quality.

Conclusions and recommendations

The consultation participants evaluated the existing evidence and arguments about the use of amino-acid-scoring patterns to evaluate protein quality, and concluded that, at present, there is no adequate basis to use different scoring patterns for different age groups with the exception of infants. Therefore, it decided to make the following recommendations:

1) The amino acid composition of human milk should be the basis of the scoring pattern to evaluate protein quality in foods for infants under 1 year of age.
2) The amino-acid-scoring pattern proposed in 1985 by FAO/WHO/UNU for children of preschool age should be used to evaluate dietary protein quality for all age groups, except infants.
3) The recommendations made here for the two amino-acid-scoring patterns to be used for infants and for all other ages must be deemed as temporary until the results of further research either confirm their adequacy or demand a revision.
4) Further research must be carried out to confirm the currently accepted values of requirements of infants and preschool-aged children, which are the basis for the scoring patterns recommended by this group of experts.
5) Further research must be carried out to define the IAA requirements of school-aged or adolescent children and of adults.

6) Given the urgency of these research needs and the magnitude of the task required, it is recommended that an FAO/WHO-coordinated international research program be immediately established to assist in the determination of human amino acid needs.

Digestibility methods

While the amino acid proportionality pattern of a protein is probably the most important determinant of protein quality, digestibility of protein and bioavailability of its constituent amino acids are the next most important factors. This is true because not all proteins are digested, absorbed, and utilized to the same extent. Differences in protein digestibility may arise from inherent differences in the nature of food protein (protein configuration, amino acid bonding), from the presence of non-protein constituents which modify digestion (dietary fiber, tannins, and phytates), from the presence of antiphysiological factors or from processing conditions that alter the release of amino acids from proteins by enzymatic processes. In recognition of this fact, in 1975, a joint FAO/WHO informal gathering of experts recommended that amino acid scores be adjusted for "true" protein digestibility.

The classic procedure for determining digestibility has been the fecal index method, an in vivo procedure in which the nitrogen excreted in the feces is subtracted from the amount ingested, and that value is then expressed as a percentage of intake. This gives an apparent digestibility value and it should be noted that the Atwater digestibility values (used in USDA's Handbook 8) developed at the turn of the century were apparent digestibility values. To determine true digestibility, it is necessary to correct for the amount of fecal nitrogen excreted when the subject is consuming either a protein-free diet, or a diet containing just enough of a highly digestible protein to prevent excessive loss of body protein.

Since TD measurements take into account the metabolic fecal nitrogen which is not of dietary origin, TD of a food is always higher than the apparent digestibility. Apparent protein digestibility values increase with increasing protein intakes, whereas TD values are independent of protein intake.

Individual amino acid digestibilities are generally determined by the fecal amino acid method, which is analogous to the determination of TD. It consists of measuring the amount of amino acid ingested in the diet, the amount excreted in the feces, and the so-called metabolic losses in the feces (estimated from the amount of amino acid excreted by an individual fed a protein-free diet) and is calculated the same way as in the determination of TD.

Protein digestibility is most frequently estimated using rats. The approach is well established and the procedure has been standardized by a collaborative study and is described in the report. Data on digestibility of protein and/or bioavailability (true digestibility) of amino acids in diets of various areas of the world, and in common foods or food ingredients have been recently reviewed. Values for true digestibility of protein from human and/or rat balance experiments in diets from India (54–75 %), Guatemala (77 %), and Brazil (78 %) were considerably lower than the

values in North American diets (including vegetarians, 88–94 %), suggesting that protein digestibility is of greater concern in the diets of some developing countries. The poor digestibility of protein in the diets of developing countries is due to the use of less refined cereals and legumes (such as beans and lentils) as major sources of protein. Low true protein digestibility values (63–65 %) have also been reported in experiments with children fed millet and ragi-based diets in India.

True digestibility studies of some common foods using human adults showed that animal protein sources (meat, fish, poultry, eggs, milk protein products), flours or breads of low-fiber wheats, wheat gluten, farina, peanuts, and soy protein isolates have high true protein digestibilities of 94–99 %, while whole corn, polished rice, oatmeal, triticale, cottonseed, soy flour, and sunflower have intermediate protein digestibility values of 86–90 %. The ready-to-eat (processed) cereals (corn, wheat, rice or oat) had low protein digestibilities of 70–77 %, caused probably by the heat involved in their processing. Millet also has a low protein digestibility of 79 %.

Advantages and shortcomings of the protein digestibility-corrected amino-acid-score method

The protein digestibility-corrected amino-acid-score method is a simple and scientifically sound approach for routine evaluation of protein quality of foods. It could be conveniently used as an additional correction factor in evaluation procedures based on both the quality and quantity of protein such as utilizable protein (g total protein × corrected score) and to replace PER in protein rating (grams protein in a Reasonable Daily Intake × PER). The amino acid score method would be the least expensive of all the suitable routines for assessing protein quality of foods, especially if the literature data for protein digestibility are used.

Unlike animal assays, which require several trials for the identification of the actual limiting amino acid, the use of the scoring procedure can readily identify the limiting amino acid in a protein source of a diet. The method also provides information about the supplementation and complementation potential of a protein source. Traditional combinations of vegetable proteins consumed in some countries (such as rice-legume in Asia, wheat-legume in the Near East, maize-legume in the Americas, etc.) have good protein quality, because the amino acid compositions of cereals and legumes complement each other, producing a balanced mixture of amino acids.

While the protein digestibility-corrected amino-acid score can be calculated for any mixture of foods from a knowledge of the digestibility and amino-acid content of the constituent foods, the score of a mixture cannot always be calculated with certainty from a knowledge of the individual scores of the components. Because of the complementary potential between proteins, a statement of utilizable protein alone for a food can be a poor indication of the utilizable protein realized when the food is consumed as part of a mixed diet. Therefore, in any consideration of nutritional labeling, the use of digestible amino acid values (especially the nutritionally important lysine, sulphur amino acids, tryptophan, and

threonine), or of total protein digestibility and amino-acid values may be preferred to a statement of the score or of utilizable protein (protein content multiplied by the corrected score). The user of the food can then calculate the corrected score for any mixture.

A further complication arises from our lack of knowledge of the proportion of the total sulphur amino acid requirement which can be met by cystine. Without that knowledge, expression of protein values in terms of the sum total of methionine and cystine has both theoretical and practical limitations.

It has been suggested that the amino-acid-score method would not take into account possible differences in absorption and utilization of amino-acid mixtures or amino-acid-supplements and proteins of the same amino-acid profile, possibly due to more rapid absorption of crystalline amino acids than the protein bound amino acids. In practice, however, this effect does not appear to be of great importance in cases involving supplementation with small quantities of amino acids.

In the case of very poor quality proteins, the amino-acid scoring approach has been criticized for non-agreement between amino-acid scores and estimates or protein quality based on biological assays. Although there is a good relationship between amino-acid score and biological assay of proteins with BV above 40 %, the agreement varies with the limiting amino acid below this level.

Another criticism of the amino-acid-score method includes its inability to take into account the possible adverse effect of disproportionate amounts of essential amino acids on the utilization of the most limiting amino acid. Excessive levels of non-essential amino acids and non-protein nitrogen may also influence the overall utilization of a dietary protein. However, the possible occurrence of amino acid imbalance in mixed or properly amino-acid-supplemented human diets does not appear to be of any major practical significance.

Summary of conclusions and recommendations

1) The consultation participants recognized that significant advancements have been made in standardizing amino-acid methodology, human amino-acid requirements, and determination of digestibility of protein and amino acids in a variety of foods.
2) They noted that methods for the determination of all amino acids in foods have been standardized, resulting in acceptable interlaboratory variation (coefficients of variation of about 10 %).
3) They recognized that the amino-acid-scoring pattern proposed in 1985 by FAO/WHO/UNU for children of preschool age is, at present, the most suitable pattern for use in the evaluation of dietary protein quality for all age groups, except infants.
4) They noted the similarity in the ability of humans and rats to digest foods, and concluded that the true digestibility of crude protein is a reasonable approximation of the true digestibility of most amino acids (as determined by the rat balance method) in diets based on animal protein sources, cereals, oilseed, legumes or mixture of protein sources.

5) They agreed that the rat-balance method is the most suitable practical method for predicting protein digestibility in humans.
6) Based on the above conclusions, they agreed that the protein digestibility-corrected amino acid score method was the most suitable approach for routine evaluation of protein quality for humans, and *recommended* the adoption of this method as an official method at the international level.
7) The consultation participants further recommended:
   - further research must be carried out to confirm the currently accepted values of protein and amino acid requirements of infants and preschool-aged children and to define the amino acid requirements of school-aged or adolescent children and of adults;
   - that FAO/WHO coordinate international research programs to determine human amino acid needs;
   - that further research be carried out to perfect and evaluate the most promising in vitro procedures for estimating protein digestibility; and
   - that FAO update the 1970 FAO publication, "Amino acid content of foods and biological data on protein" with reliable amino acid data, and commission new analyses of foods where there are insufficient reliable data.

Conclusions

The protein digestibility-corrected amino-acid score is considered the most suitable regulatory method for evaluating protein quality of foods and infant formulas. Since this method is based on human amino acid requirements, it is inherently more appropriate than animal assays used for predicting protein quality of foods and the consultation participants therefore recommend that the procedure be adopted as the preferred method of measuring protein values in reference to human nutrition.

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