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Protein quality evaluation

Report of Joint FAO/WHO Expert Consultation

FAO FOOD AND NUTRITION PAPER

51

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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Report of the Joint FAO/WHO
Expert Consultation
Bethesda, Md., USA
4-8 December 1989

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REPORT OF A JOINT FAO/WHO EXPERT CONSULTATION ON

PROTEIN QUALITY EVALUATION

Bethesda, MD, USA, 4-8 December 1989

1. BACKGROUND FOR THE CONSULTATION

The First session of the Codex Committee on Vegetable Proteins (CCVP) was held in Ottawa on November 3-7, 1980. The Committee, while elaborating general guidelines for the utilization of vegetable protein products in foods, felt the need for a suitable indicator to express protein quality. They discussed the subject of protein equivalence and pointed out that Protein Efficiency Ratio (PER) might not be the most suitable means for protein quality evaluation.

At its second session (Ottawa,March 1-5, 1982), the CCVP considered for adoption the Relative Net Protein Ratio (RNPR) (a rat assay procedure) as an indicator for protein quality, but deferred the decision since several delegations at the session were of the opinion that insufficient research data were as yet available to establish the comparative values of some of the methods discussed.

At its third session (Ottawa, February 6-10, 1984), the CCVP considered the suitability of using amino acid composition data (amino acid scores) corrected for crude protein digestibility/amino acid availability as a measure of protein quality. It did not take any decision but agreed to continue its studies.

The Committee at its fourth session (Havana, February 2-6, 1987), noted improvements made in amino acid methodology and amino acid requirement pattern since its last session, and discussed initial data from ongoing USDA-organized cooperative studies involving amino acid availability, nitrogen digestibility and protein nutritional assessment based on amino acid composition data. The Committee concluded that an amino acid scoring procedure, corrected for true digestibility of protein and/or bioavailability of limiting amino acids, is the preferred approach for assessing protein quality of VPP and other food products. However, an official recommendation was deferred because i) there was a need to standardize and collaboratively test in vitro methods for predicting protein digestibility of foods to be used for correcting amino acid scores, and ii) further improvement in amino acid methodology, especially for determination of tryptophan was desired.

Based on results of collaborative studies undertaken in 1987 and 1988 (1-3) to address the above issues, and on recent improvements in amino acid methodology, the CCVP at its fifth session (Ottawa, February 6-10, 1989) endorsed the use of the 1985 FAO/WHO/UNU suggested pattern of amino acid requirements of a two to five year-old child (4) 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 (5). 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 review the issues. Such a Consultation should be requested to review the results of studies carried out by the ad hoc Working Group on Protein Quality Measurement (Coordinated by Dr. G. Sarwar, Canada) and evaluate the protein digestibility-corrected amino acid score method for its usefulness for evaluating protein quality in human nutrition.

2. INTRODUCTION

A Joint FAO/WHO Expert Consultation on Protein Quality Evaluation was held in Bethesda, MD from December 4 to 8, 1989. The provisional agenda adopted by the Consultation is attached as Annex 1. The membership of the Consultation is given in Annex 2.

Dr. William H. Tallent, Assistant Administrator, Agricultural Research Service, U.S. Department of Agriculture, and head of the U.S. delegation to the CCVP, welcomed the participants to the meeting. He expressed the wish that the important work of the Consultation would be successful and that the participants would enjoy their time in Washington.

Mr. Roger A. Sorenson, Head, FAO Liaison Office for North America, extended a warm greeting to the experts on behalf of the Directors-General of FAO and WHO and thanked the U.S. government for its generous offer to host the Consultation and in particular the United States Department of Agriculture (USDA) and the Agricultural Research Institute for their cooperation and support in organizing the meeting. He recalled that the meeting was a direct follow-up to a recommendation by the CCVP at its fifth session held in Ottawa from February 6 to 10, 1989. He also underlined 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. He reported that higher protein quality products can constitute a good source of foreign currency for many developing countries. He emphasized that the effort deployed by the CCVP in developing world-wide standards and guidelines help eliminate non-tariff barriers and facilitate international trade of vegetable protein products. He hoped that the results of this Consultation will further strengthen these efforts.

Dr. E. Boutrif of the Joint FAO/WHO Secretariat made a brief introduction in which he reminded the participants of the objectives of the meeting, which 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, i.e., amino acid score corrected for digestibility.

The Consultation designated Dr. Peter Pellett as Chairman and Dr. Bjorn Eggum as Vice-Chairman. Dr. Eric Miller was appointed as rapporteur.

3. GENERAL CONSIDERATIONS

3.1 Regulatory Needs in Assessing Protein Quality of Human Foods

From a regulatory perspective, both public health needs and the economic impact of protein quality characterization are important in the selection and approval of methods for assessing protein quality. The public health needs for assessing protein quality are well established. Humans require certain minimal quantities of essential amino acids from a biologically available source as part of a larger protein/nitrogen intake. The required amounts of these amino acids vary with age, physiological condition and state of health. The economic considerations are derived primarily from the need to discriminate with both accuracy and precision the relative efficiency with which individual protein sources can meet human biological needs.

It is widely recognized that clinical human studies which measure growth and/or other metabolic indicators including nitrogen balance provide the most accurate assessment of protein quality. For reasons of both cost and ethics, it is considered inappropriate to routinely measure protein quality through the use of such techniques. Consequently, assay techniques designed to measure the effectiveness of a protein in promoting animal growth have been utilized. Since 1919, the Protein Efficiency Ratio (PER) method, which measures the ability of a protein to support growth in young, rapidly growing rats, has been used in many countries because it was believed to be the best predictor of clinical tests. However, after decades of use, it is now known that PER over-estimates the value of some animal proteins for human growth while under-estimating the value of some vegetable proteins for that purpose. The rapid growth of rats (which increases the need for essential amino acids) in comparison to human growth rates is the reason for this discrepancy. This discrepancy results in an economic rather than a public health problem because PER generally errs on the side of safety.

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 following should be considered as criteria for assessing the suitability of this combination of amino acid score and protein digestibility in predicting protein quality:

1. The methods used should provide results which are consistent with results from clinical studies designed to assess protein quality.
2. Any inconsistency between proposed methods and results from clinical studies should err on the side of safety.
3. The methods should be applicable to the entire range of foods used in human diets.
4. Results from collaborative studies should demonstrate excellent repeatability within a laboratory and reproducibility between laboratories.

5. The methods should not require unreasonably large or unreasonably small samples and questions of homogeneity become more important as samples size decreases.

6. The methods should permit the assay to be accomplished on the finished product (i.e., on the form consumers purchase).

3.2 Economic Considerations

In order to place these considerations in economic perspective, world production and trade in protein foodstuffs, of both animal and plant origins, have significantly increased during the last decade as a result of a galloping demand due to an increased world population. In 1984, the total world production of animal protein foodstuff reached 1745.6 million metric tons (184.7 million metric tons of animal protein) while that of plant protein foodstuffs was estimated to amount to 2447.3 million metric tons (249.9 million metric tons of vegetable protein).

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 food 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. Protein quality measurement can also have secondary effects which may be as costly to populations and governments by stimulating the high consumption of food which affects health and disease.

The methods presently used for measuring protein value of foods were established when information was not extensively available on human amino acid requirements. Therefore, while results were produced which were "safe," they did not accurately reflect human 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 does the human (Table 1). In addition to the higher requirement for sulfur amino acids, 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 acid requirements of the rat. Table 1 shows that casein provides only 70-87% of the sulphur 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 sulfur amino acid content of casein.

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

Table 1. Essential amino acid requirement and content, mg/g protein.

	A	B	C	D	E
Essential Amino Acid	2-5 Year[a] Child	Laboratory[b] Rat	Casein[c]	70% of Rat Req.	Ratio of 70% Rat Req. to Human Req.
Arginine	—	50	37	35	—
Histidine	19	25	32	18	0.92
Isoleucine	28	42	54	29	1.05
Leucine	66	62	95	43	0.65
Lysine	58	58	85	41	0.70
Methionine & Cystine	25	50[d]	35	35	1.40
Phenylalanine & Tyrosine	63	66	111	46	0.73
Threonine	34	42	42	29	0.86
Tryptophan	11	12.5	14	10	0.89
Valine	35	50	63	44	1.26

[a]FAO/WHO/UNU, 1985. (4)
[b]National Research Council (6), based on a protein requirement of 12% plus an ideal protein (100% true digestibility and 100% biological value).
[c]Steinke, et al, 1980. (7)
[d]A lower rat requirement of 40 mg/g protein for methionine & cystine has also been reported (124).

selection of food and in economic loss to consumers and producers. This can be avoided by directly comparing food proteins to human amino acid patterns.

There are numerous restrictive national policies based on meeting a specific PER value which, in developed countries, has resulted in increased costs of foods to the general population with no perceivable benefit. In 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 programmes may have vital significance.

A major effort is presently underway in many countries to modify dietary patterns to aid in the prevention of chronic diseases and particularly heart disease (8,9,10). These dietary recommendations include reduction in the consumption of saturated fats. Thus the emphasis is now on developing designed foods which have less animal products and more vegetable foods in the diet to help reduce blood cholesterol.

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 while reducing animal fats in the diet to provide the consumer with a better and more economical food selection since animal products in general are higher in cost per unit of protein (11).

4. SCIENTIFIC BASIS FOR THE ADOPTION OF THE PROTEIN DIGESTIBILITY-CORRECTED AMINO ACID SCORE METHOD

The fundamental measurement of protein quality for human use depends on growth and/or other metabolic balance evaluation procedures performed on suitable subjects of the target population. Those procedures directly reflect the essential (indispensable) amino acid content, digestibility of the protein, and bioavailability of the amino acids in a food or food product. Recognizing that such tests require 35-45 days and cost from $12,000-18,000 per subject, and that such studies cannot be done on a routine basis in humans, it is necessary to develop in vitro or animal assay techniques which correlate closely with data from human experiments.

Rat growth assays have been widely used for predicting protein quality in foods, and numerous workers have discussed the appropriateness of these methods [(See review in Sarwar & McDonough (12)]. The most serious problem with the rat growth assay is the higher requirements of rats for some amino acids when compared to humans. The Protein Efficiency Ratio (PER = weight gain of test group/protein consumed by test group) is the official method for assessing protein quality of foods in Canada and the United States, but it has been severely criticized for not meeting the criteria for a valid routine test (12). A major criticism of the PER assay is its inability to properly credit protein used for maintenance purposes. A protein source may not support growth and have a PER near zero, yet still may be adequate for maintenance purposes. Due to the error introduced by not making allowance for maintenance, the PER values of proteins of differing quality are not proportional (in protein quality) to each other, i.e., a PER of 2.0 cannot be assumed to be twice as good as a PER of 1.0. The lack of proportionality to protein quality makes the PER method unsuitable for the calculation of utilizable protein, such as in protein rating (protein in a reasonable daily intake, g X PER), which is the official method of evaluating protein claims of foods sold in Canada. The PER and other methods were reviewed at the Airlie Conference in 1980, where it was agreed that the PER should be replaced by a more appropriate and precise method (13).

The nutritive value of a protein depends upon its capacity to provide nitrogen and amino acids in adequate amounts to meet the requirements of an organism. Thus, in theory, the most logical approach for evaluating protein quality is to compare amino acid content (taking bioavailability into account) of a food with human amino acid requirements. A number of comparisons have been made using reference patterns such as those derived from egg or milk protein. The first major change in procedure was substitution of a provisional pattern of amino acid requirements for the egg protein standard. A hypothetical reference protein derived from the pattern of human amino acid requirements was proposed as the standard for comparison.

Shortcomings have been recognized and progress has been made in accurately evaluating human amino acid requirements. Equally critical for success is the ability to obtain precise measurements of amino acid content in the test protein sources. Finally, to improve on accuracy of scoring procedures, chemically determined amino acid content may have to be corrected for digestibility or biological availability.

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 many cases, the only practical approach.

5. AMINO ACID ANALYSIS

5.1 Review of Principal Methods of Analysis of Amino Acids

The main methods involve acid or alkaline hydrolysis of the protein followed by separation and quantification of the released amino acids by ion-exchange (IEC), gas-liquid (GLC) or high performance liquid chromatography (HPLC). Many variants exist of each of these main classes. Other chemical and microbiological methods are available for specific amino acids, e.g. lysine, methionine, cystine, and tryptophan. In some cases, the specific methods do not require prior acid or alkaline hydrolysis. Such methods may be valuable in specific investigations and could be used in chemical scoring procedures where the limiting amino acid is already known. However, the development of rapid methods for the analysis of all the amino acids suggests less use will be made of the specific methods.

5.1.1 *Hydrolysis*

The most usual method of hydrolysis is with 6 M HCl either in evacuated sealed tubes at 110° +/- 0.5° C or refluxing under a stream of nitrogen for 22-24 hr. Effects of varying hydrolytic conditions have been reviewed (14-19).

Under these conditions cyst(e)ine and tryptophan are largely destroyed, so separate analyses must be made for these two amino acids. Similarly, while methionine can be determined in protein rich foods when care is taken to exclude oxygen, there are considerable losses with carbohydrate-rich foods (16). Methionine is best determined along with cyst(e)ine in performic acid oxidized protein. Threonine and serine also suffer partial destruction. In contrast, valine and isoleucine are not completely released after 22-24 hr. In very precise work, several hydrolysis times have been used in order to extrapolate to maximal values for threonine, serine, valine and isoleucine. Under defined conditions of time and temperature, correction values for incomplete recovery may be applied. An example of the factors used at one national research center (TNO, The Netherlands) for 22 hr hydrolysis at 110° C are threonine 1.05, serine 1.10, valine 1.07 and isoleucine 1.08.

Shorter hydrolysis times at higher temperatures have also been used. Gehrke et al. (16) found 4 hr at 145° C to be satisfactory with close agreement overall with values obtained after 24 hr at 110° C, but threonine and serine had values 7% and 13% less, respectively while valine and isoleucine were 8 and 9% greater. Correction factors appropriate to the specific hydrolytic conditions must be used for these amino acids.

Hydrolysis with organic sulphonic acids has been used to measure tryptophan and all other amino acids in pure proteins but in the presence of carbohydrate, tryptophan is destroyed (20,21). The method has been used to determine the methionine sulphoxide content of foods (22). Very short time (45 minutes) at high temperature (160° C) has also been used to hydrolyze all amino acids including cystine and tryptophan in pure proteins. Further research on such methods for the analysis of foods

is desirable, but at the present state of knowledge, use of organic sulphonic acids is not recommended for routine use.

The first action AOAC method for cystine and methionine requires performic acid oxidation prior to hydrolysis (23). The presence of large amounts of chloride, as sodium chloride at 1 to 7 times the methionine content, significantly reduces the recovery of methionine as methionine sulphone. The interference can be eliminated by adding water to the performic acid reagent but, under the changed conditions, cystine recoveries are incomplete (24). Special care needs to be taken to adjust the oxidation conditions when analysing salt-rich foods. Andersen et al. (25) summarized a series of studies on hydrolytic conditions for oxidised proteins but more recent revisions of their oxidation and hydrolysis procedure have been proposed (22). The oxidised hydrolysate can also be used for the determination of all other amino acids except for tryptophan, tyrosine, phenylalanine and histidine. The time of hydrolysis of oxidized samples may also be reduced to 4 hr at 145° C without loss of lysine but with increased loss of threonine (27).

Alkaline hydrolysis is currently used for tryptophan analysis in foodstuffs. Barium hydroxide, sodium hydroxide, and lithium hydroxide can be used provided appropriate precautions described in the literature are followed. Barium hydroxide needs a precipitation step before chromatography and tryptophan loss during this procedure by absorption and occlusion must be prevented (28). With all three reagents it is necessary to remove oxygen before hydrolysis. This can be achieved by boiling the reagents before samples are added (29,30) by using evacuated tubes, or by flushing with nitrogen (31). Variable hydrolysis times and temperatures have been proposed with 4.2 M sodium hydroxide for 16 or 20 hr at 110° C, with barium hydroxide (8.4 g Ba[OH]2.8 H_2O plus 16.0 ml water) for 7-8 hr at 120-130° C (25).

Tryptophan losses of 10 to 20% occur during hydrolysis. When internal standards such as alpha-methyl-tryptophan or 5-methyl-tryptophan are used during hydrolysis, the use of a correction factor can be eliminated, because the internal standard will be decomposed to a similar extent to that of protein-bound tryptophan (30,31).

5.1.2 *Ion-exchange chromatography (IEC)*

Commercial equipment is available for quantitative analysis of amino acids according to the classical ion-exchange procedures of Spackman, Stein and Moore (32) and Hamilton (33). Experienced personnel are required to use this equipment successfully and meticulous attention to detail is required to achieve accurate and reproducible results. The method has been reviewed by Blackburn (14). Eluted amino acids are usually measured by reaction with ninhydrin and spectrophometric determination at 570 nm for alpha amino acids and 440 nm for the amino acids proline and hydroxyproline. Although this procedure is still the main one in use, the faster and better separations possible with GLC and HPLC are tending to supersede classical IEC.

5.1.3 *Gas-liquid chromatography (GLC)*

This requires conversion of the amino acids to volatile derivatives. Successful quantitative conversion and separation has been achieved using N-trifluoracetyl-n-butyl esters (34), N-heptafluorobutyryl-isobutyl esters (35) and tert-butyldimethylsilyl derivatives (36-40). Although each of these methods has been applied to acid hydrolysates of protein, none has been applied to oxidised or alkaline hydrolysates for the determination of sulphur amino acids or tryptophan.

5.1.4 *High-performance liquid chromatography (HPLC)*

The use of HPLC in amino acid analysis is reviewed by Williams (18). HPLC may be used to separate amino acids on ion-exchange columns with post-column derivatization with ninhydrin or OPA (41), or by pre-column derivatization followed by separation on reversed phase octyl or octadecyl silica. The advantages of pre and post-column derivatization have been reviewed by Engelhardt (42) and Cohen & Strydom (43). With the availability of rapid and easily automated methods of pre-column derivatization and the lower cost of such systems compared with post-column derivatization, the pre-column derivatization is to be preferred. Recently commercial systems based on this methodology have been marketed.

Pre-derivatization with phenylisothiocyanate is described by Bidlingmeyer et al. (44); with o-phthaldialdehyde (OPA) by Jones and Gilligan (45); with 9-fluorenylmethyl chloroformate (FMOC-Cl) by Einarsson et al. (46); with 1-fluoro-2,4-dinitrobenzene (FDNB) by Morton and Gerber (47); with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (DNPAA) by Kochhar and Christen (48); and with dansyl chloride by Thio & Thompkins (49). Some of the features of the methods are summarized in Table 2.

5.2 Comparison of results by the different methods

A number of studies of GLC and HPLC methods have compared mean results obtained with values determined by IEC taken as the standard. Most comparisons have involved pure proteins. Some have compared the determined number of residues per mole of protein with the theoretical value obtained by complete sequencing. Where comparable hydrolytic conditions are used results by the various methods have usually been in good agreement. Where estimates of within laboratory variability have been given, the newer methods have appeared to have similar precision to IEC. For example, Gherke et al. (34) report the analysis on the same hydrolysates of 7 foods and 1 pure protein as 1.95 percent less (SEM 0.93) for lysine and 0.62 percent more (SEM 0.90) for threonine by GLC of trifluoroacetyl-n-butyl esters than values by IEC. Bidlingmeyer et al. (44) reported HPLC of PTC amino acids agreed well with IEC. In the example given lysine was 13 percent greater by HPLC while threonine was identical with IEC values for one feed sample. Coefficients of variation for lysine and threonine by the PITC method were 2.8 and 1.8 percent, respectively. Closely similar variability values for lysine and threonine of 2.9 and 1.7 percent, respectively, were reported by Sarwar et al. (50) using essentially the same HPLC method; in addition, values for methionine and cystine after performic

Table 2. Summary of methods suitable for precolumn derivatization

	PITC	OPA	FMOC	FDNB	FDNPAA	DANSYL
Derivatization time (min)	20	0.5	5	30	50	30
Removal of reagent by drying	YES	NO	NO	YES	YES	NO
Solvent Extraction	NO	NO	YES	NO	NO	NO
Determines sec-amine	YES	NO	YES	YES	YES	YES
Quantitative yield	YES	YES	YES	YES	YES	YES
Stable derivative	YES	NO	YES	YES	YES	NO
Interfering side products	NO	NO	NO	YES	YES	YES
Detection	254nm	Fluor	Fluor	365nm	340nm	Fluor
Sensitivity	fmol	pmol	fmol	pmol	pmol	pmol
Interference by contaminants in eluant	YES	NO	NO	NO	NO	YES
Chromatogram run time (min)	15	18	30	70	110	30

Abbreviations

DANSYL	5-dimethylamino-1-napthalenesulfonyl chloride
FDNB	1-fluoro-2,4-dinitrobenzene
FDNPAA	1-fluoro-2,4-dinitrophenyl-5-L-alanine amide
FMOC	9-fluorenylmethyl chloroformate
OPA	o-phthaldialdehyde
PITC	phenylisothiocyanate

acid oxidation were 2.7 and 3.3 percent, respectively. Tryptophan in alkaline hydrolysate was separated by HPLC and measured in the UV without derivatization with a coefficient of variation between hydrolysates of 4.0 percent. In the method of Nielsen and Hurrell (31) for tryptophan, the coefficient of variation for single determinations was 2.3 percent compared with 5.4 percent for the colorimetric procedure of Miller (28) and 7.6 percent for the spectrofluorometric procedure of Buttery and Soar (51). The more sensitive FMOC method appears to be possibly more variable. Einarsson et al. (46) reported FMOC values for lysine, methionine, threonine and cysteic acid. Coefficients of variation of the derivatization and chromatography (but excluding hydrolysis) were 3.3, 4.5, 4.8 and 5.0 percent, respectively. Duodenal digesta samples analysed by performic acid oxidation (52), hydrolysis and FMOC derivatization gave coefficients of variation for lysine, methionine sulphone, cysteic acid and threonine of 5.1, 2.1, 2.3 and 1.7 percent, respectively (53). The overall conclusion is that each of the methods reviewed is capable of giving results, within a laboratory that is skilled in the technique, with repeatability within the range 2 to 5 percent. However, as shown in the next section, greater variability is obtained when the results of different laboratories are compared.

5.3 Results of collaborative trials

Early collaborative trials were summarized by Williams (54). Mean and ranges of coefficients of variation of laboratory means for the key amino acids in standard amino acid mixtures and in proteins determined by IEC are given in Table 3.

Table 3. Coefficients of variation of laboratory means in collaborative trials of amino acid analysis by ion-exchange chromatography from Williams (54)

	Coefficients of Variation (%)						
	Amino acid standards			Proteins			
	Mean	Range	N1	Mean	SD.	Range	N2
Lysine	4.43	2.5-7.9	4	11.2	6.8	2.1-20.0	18
Methionine	9.13	5.7-12.6	4	20.9	17.4	0.0-61.2	18
Cystine	2.8	-	1	18.3	10.2	6.4-46.2	13
Threonine	4.98	1.8- 8.1	4	12.1	6.2	3.4-23.2	18
Tryptophan	-	-	-	42.6	55.1	13.8-141.1	5

N1 Number of trials; N2 Number of proteins studied in 8 trials.

These early trials included use of manual systems as well as early automatic instruments. The variability of analysis of standard solutions were no better than values obtained in microbiological assays of 6.9, 2.4, 4.8, 11.0 percent for lysine, methionine, threonine and tryptophan, respectively. Part of this variation can be attributed to differences in local standard solutions and sample dilution and preparation errors. Variability in the analysis of proteins by IEC was considerably greater, implicating hydrolysis as the major additional source of error.

Results of more recent collaborative studies of IEC are given in Table 4. Sarwar et al. (55) analysed seven foods in seven laboratories. All laboratories determined cysteic acid in performic acid oxidised hydrolysates, five laboratories also measured methionine as the sulphone while the remaining two laboratories determined methionine in the unoxidised hydrolysate. Six laboratories determined tryptophan in 4.2N NaOH hydrolysates by IEC and the seventh by the Spies and Chambers method using p-aminobenzaldehyde on the intact protein. Each laboratory carried out each analysis in duplicate. The results were presented as coefficients of variation within laboratory for the means of duplicate determinations. Since coefficients of variation are defined on the basis of single determinations the data given by Sarwar et al. (55) have been corrected to a single determination basis so as to be comparable with other studies. Their report does not mention the exclusion of any submitted data from statistical analysis. The separate estimates for the individual foods have been combined by taking the mean of the squares of the coefficients of variation and then taking the square root to obtain the mean coefficient.

Table 4. Estimates of coefficients of variation within and between laboratories in Ion Exchange analysis of amino acids.

	Coefficients of Variation (%)								
	Standards		Proteins						
	Andersen et al (25)	Miller et al (56)	Sarwar et al (55)		Andersen et al (25)		Miller et al (56)		McDonough et al (57-59)
	B	B	W	B	W	B	W	B	B[a]
Lys	5.4	7.6	3.2	7.5	2.9	8.5	6.1	10.8	6.0
Met	6.8	11.0	4.4	11.7	3.5	7.7	6.9	13.6	13.4
Cys	5.5	-	4.3	14.6	5.1	9.0	-	-	20.3
Thr	6.0	7.1	3.2	8.2	3.8	5.8	7.3	7.4	-
Trp	-	-	3.7	19.1	-	-	-	-	16.5

[a]Reproducibility underestimated as based on mean of duplicate determinations within each laboratory; see text.
W: Within laboratory variation (repeatability)
B: Between laboratory variation (reproducibility)

Andersen et al. (25) organized a trial in which 30 laboratories in the EEC tested a newly developed hydrolysis procedure. Each laboratory was sent standard solutions and 10 feed samples representing hidden duplicates of 5 feedstuffs. Twenty-three laboratories returned data analysing the circulated standard against their own standard.

Results from one laboratory were rejected and a further two values for each of cyst(e)ine, proline, serine and valine were rejected. Twenty-five laboratories returned values for the feedstuffs from which 27 percent of the data was rejected, the total rejection of data from 4 laboratories accounting for 15-16 percent of the available data. Tryptophan was not determined.

Miller et al. (56) reported a study in which standard solutions and hidden duplicates of eight fishmeals were analysed by five laboratories by IEC and by three laboratories using GLC. The IEC results only are shown in Table 4. Three laboratories determined the sulphur amino acids in oxidised hydrolysates, one determined methionine in the unoxidised hydrolysate and one determined methionine as an iodoplatinate complex. Tryptophan was not determined. On inspection of the submitted data, a systematic trend in the differences between hidden duplicates from one laboratory in data for the unoxidised hydrolysates became apparent (the laboratory also prepared oxidised hydrolysates for the analysis of sulphur amino acids). The laboratory concerned believed this was due to ageing of the ninhydrin reagent and subsequently withdrew their data from the trial on the basis that it was not representative of normal operation. Other outlying values were identified in the remaining data. After inspection and correction of some of these, 17 outlying values remained out of a total of 1744 determinations. These were included in the final analysis.

McDonough et al. (57-59) reported collaborative studies on lysine, methionine, cystine and tryptophan bioavailability which necessitated determination of total amino acids by IEC. Five laboratories participated. Performic acid oxidation was used for the sulphur amino acids. Tryptophan was determined by IEC in 4.2N NaOH hydrolysates. Full details of procedures are not given but each laboratory analysed each of 17 foods in duplicate. Only means and the SD of the 5 mean values reported by the cooperating laboratories are given. It is not possible to adjust these data to give the correct estimate of reproducibility (between laboratory variability). The error mean square corresponds to

$$\text{EMS} = \frac{\sigma_e^2}{2} + \sigma_L^2$$

where σ_e^2 is the variance between duplicate determinations in the same laboratory and σ_L^2 is the variance due to differences between laboratories. Reproducibility standard deviation for the analysis of any one food is defined (56) as:

$$\sigma_x = \sqrt{\sigma_e^2 + \sigma_L^2}$$

The coefficient of variation based on means of duplicate determinations therefore underestimates reproducibility by including only half the variance attributable to within laboratory variation. However, since this is usually very much smaller than the between laboratory variation, reproducibility is not greatly underestimated. The mean coefficient of variation was obtained for the 17 feeds via the mean of the squares of the coefficients for the separate foods.

No collaborative studies of GLC analysis other than that of Miller et al. (56) have been reported and the latter only involved three laboratories. Only unoxidised hydrolysates were analysed. Neither cystine nor tryptophan values were reported and methionine was determined in unoxidised hydrolysates. The coefficients of variation for repeatability and reproducibility for lysine were 5.1 and 4.8 percent respectively, and for methionine 4.6 and 8.2 percent. MacDonald et al. (61) reported a collaborative study of the determination of sulphur amino acids in which 7 laboratories used the 1985 AOAC method (23) to analyse 6 foods and a pure protein. One laboratory was unable to obtain sufficient resolution of methionine sulphone. Otherwise no other data were eliminated on the basis of outliers. The repeatability and reproducibility coefficients of variation pooled over the 6 foods are compared with those for the pure protein in Table 5.

A collaborative study specifically of the determination of sulphur amino acids and tryptophan by the AOAC methods has recently been published (62). Nine laboratories took part; seven analysed the sulphur amino acids, all by ion-exchange but one laboratory used HPLC equipment. Seven laboratories determined tryptophan in 4.2N NaOH hydrolysates, three by reverse phase HPLC and four by IEC. Laboratories analysed 6 foods and 1 pure protein in duplicate. In addition, each laboratory analysed once, 3 centrally prepared oxidised acid hydrolysates of foods. The submitted

data were examined for chromatographic separation and statistical validity. About 9 percent of the cystine data, 4 percent of the methionine and 18 percent of the tryptophan data were rejected.

Table 5. Estimates of coefficients of variation within and between laboratories in a study of the AOAC method for sulphur amino acids (61).

	Coefficient of Variation (%)			
Amino Acid	Feedstuffs		Pure Protein	
	W	B	W	B
Met	5.1	8.9	1.9	2.3
Cys	4.3	8.8	2.5	2.7

W: Within laboratory variation (repeatability)
B: Between laboratory variation (reproducibility)

The tryptophan data from one laboratory were rejected because lack of detector sensitivity resulted in poor chromatographic peaks that were not integrated by the data acquisition system. The results are summarized in Table 6. Mean values for the foods and centrally prepared hydrolysates were calculated via the mean of the squares of the individual coefficients of variation.

Table 6. Estimates of coefficients of variation within and between laboratories in a study of AOAC methods (62).

	Coefficients of Variation (%)				
Amino Acid	Hydrolysates	Feedstuffs		Pure Protein	
	B	W	B	W	B
Met	5.5	6.2	8.7	3.2	8.0
Cys	7.2	4.6	13.9	4.1	9.9
Trp	-	9.9	10.9	4.6	16.5

W: Within laboratory variation (repeatability)
B: Between laboratory variation (reproducibility)

The within- and between-laboratory variation for the pure protein were within the range of values for the foods. The variation between laboratories for the prepared hydrolysates was closely similar for the three hydrolysates and generally less than obtained when the laboratories prepared their own hydrolysates. It was, however, comparable to the within-laboratory variability. This indicates that while hydrolytic conditions within a laboratory can be well standardized, larger differences occur between laboratories as a result of carrying out the same procedure.

5.4 Recommended procedures

5.4.1 *For determination of all amino acids*

1. Three hydrolysates, in duplicate, are required:

i. acid hydrolysis of unoxidised protein for determination of all amino acids except tryptophan, methionine and cystine;

ii. acid hydrolysis of oxidised protein for determination of methionine and cystine; this hydrolysate may also be used for the determination of all other amino acids except tryptophan, tyrosine, phenylalanine and histidine; values for amino acids determined in both acid hydrolysis i) and ii) may be averaged;

iii. alkaline hydrolysis of unoxidised protein for tryptophan.

2. Acid hydrolysis of unoxidised and oxidised protein should be conducted according to the detailed protocols given by Pellett and Young (63), Mason et al. (52), AOAC (64), AOAC (23), or Finley (17), with the exception that oxygen should be rigorously excluded during unoxidised hydrolysis but no such precautions are necessary with oxidised protein. Similar procedures, with respect to sample weight, acid volume, temperature and time of hydrolysis should be followed. Considerable differences exist between the above published methods in these latter conditions. Nevertheless, these methods have been widely used and collaborative trials have given adequate reproducibility for routine evaluation. Further improvements in precision and reproducibility may be achieved if critical experimental evaluation of these alternative techniques is undertaken, including studies with proteins of known composition.

Main features of a satisfactory method should include:

i. Sufficient sample size to ensure good sampling.

ii. A minimum of 100 ml 6 M HCl per 1g food dry matter, although a wide range of acid and sample may be used without appreciable differences.

iii. Removal of oxygen from unoxidised hydrolysates by freezing and evacuation to 50 m Hg or less, allowing to thaw under vacuum and repeating the cycle twice more before sealing under vacuum; or evacuating as above, flushing with nitrogen before sealing the tube or stoppering the flask; or by conducting the reflux hydrolysis under a continuous flow of nitrogen.

iv. Hydrolysis time at 110° +/- 0.5° C of 22 hr is optimal and allows for a daily schedule of hydrolysate preparation.

v. Neutralization of the hydrolysate where possible rather than rotary evaporation; alternatively, where subsequent procedures require minimal HCl or salt, rotary evaporation at temperatures not greater than 40° C. Temperature may be a critical factor where the sample is taken to complete dryness rather than concentrated to a small volume (1 ml) (65).

3. Oxidation of protein should be carried out using performic acid prepared and used as described by Moore (66), Pellett and Young (63), Mason et al. (52) or AOAC (23). The ratio of performic acid to protein in these procedures is in the range 0.08 - 1.3 ml/mg crude protein. The procedure detailed in AOAC (64) is not recommended as the amount of performic acid used is considerably less than in the recommended methods.

4. Alkaline hydrolysis should be carried out by one of the procedures described by Slump and Schreuder (29), Pellett and Young (63), AOAC (64), Nielsen and Hurrell (31), Finley (17) or Bech-Andersen (30). Differences in detail exist between these methods, e.g., extent of vacuum necessary to remove oxygen, use or omission of starch as a reducing agent, use of lactose instead of starch, use of polypropylene liners instead of Pyrex glass, boiling or steaming in an autoclave to remove oxygen, some of which may warrant further investigation. The use of 5-methyl-tryptophan or alpha-methyl-tryptophan as an internal standard carried through the entire procedure is recommended. Conflicting reports exist as to which is the better indicator of destruction of protein-bound tryptophan. Further research on this aspect is required.

5. Amino acids in the hydrolysates should be determined by classical IEC, by HPLC using cation exchange resins and post column derivatization or by prederivatization followed by reverse phase HPLC. In the latter case, derivatization and separation procedures which have been shown to give results with foods equivalent to classical IEC should be used. The PITC method has been found satisfactory in this respect. Comparative results with other derivatization reagents are required. Collaborative tests of the HPLC methods should be undertaken.

6. Tryptophan and the internal standards 5-methyl-tryptophan or alpha-methyl-tryptophan are best separated by reverse phase HPLC and quantified by UV absorption or fluorescence without derivatization.

7. Results should be expressed as mg amino acid/g N. Results for threonine, serine, valine, and isoleucine should be corrected for hydrolytic losses by factors based on time-hydrolysis studies conducted once in each laboratory. Recovery of methionine and of cystine as methionine sulphone and cysteic acid, respectively, should be determined and correction factors applied as necessary. A correction factor for loss of tryptophan should be applied if the internal standard method is not used.

8. A protein of known composition should be regularly analysed to test for variability in the analytical procedures.

9. It is desirable that where possible the recovery of nitrogen from amino acids and ammonia be calculated as a check on the quality of the analysis. For most food products the recovery of nitrogen should be greater than 90 percent. For foods known to contain significant amounts of non-amino-acid nitrogen, such as yeast rich in nucleic acids, recoveries of nitrogen may be in the range 80 to 85 percent.

5.4.2 *Partial amino acid analysis*

In the great majority of cases the nutritionally important amino acids are lysine, methionine/cystine, tryptophan and threonine. Analysis of these amino acids alone may provide sufficient data for calculating amino acid score. Tryptophan may be determined by alkaline hydrolysis and IEC or HPLC as discussed earlier, but where this equipment is not available the colorimetric procedures of

Miller (28) and Buttery and Soar (51), which have been shown to give comparable values to the HPLC method (31), may be used.

The remaining amino acids may be determined using performic acid oxidation and hydrolysis as described earlier. Where IEC or HPLC equipment is not available, specific methods for lysine e.g. lysine decarboxylase, for cystine e.g., using Ellman's reagent and for methionine e.g., using sodium nitroprusside (67) do exist and may be used when results have been shown to be comparable to values obtained by the chromatographic methods.

5.4.3 *Use of published amino acid data*

A review of a 1970 FAO publication on amino acid content of foods (68) and of a number of other national food composition tables reveals considerable shortcomings in the FAO data and considerable variability between values reported in the national tables, especially for tryptophan, cystine and methionine. It is recommended therefore, that FAO compile a new table of reliable amino acid data obtained by modern techniques according to the specifications outlined in this report and that new analyses of foods be commissioned when there are insufficient reliable data. When reliable tables of tightly specified products exist, the data may be used for the calculation of amino acid score.

5.5 Conclusions and Recommendations

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 acid 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 (68) 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.

6. AMINO ACID SCORING PATTERN

6.1 Background

The use of amino acid composition data for the evaluation of protein values of foods and diets has been widely used since the amino acid composition of egg was introduced as a standard by Block and Mitchell (69). This procedure was adopted by FAO in 1957 (70) and, with some further modification also, in 1965 (71). The high levels of indispensable (essential) amino acids (IAA) in egg proteins gave relatively low amino acid scores for many food proteins and so, subsequently, human amino acid requirement values served as the basis for several amino acid scoring systems (4,72). Although the 1974 and 1980 NAS-NRC scoring procedures (73,74) were also claimed to be based on human amino acid needs, in practice the pattern was derived from the amino acid composition of egg and milk proteins. This explains why the scoring pattern proposed by the U.S. group (73) differed from the 1973 FAO/WHO (72) values, especially for total sulphur amino acids. In 1973, FAO/WHO (72) proposed a provisional scoring pattern based on the experience gained from using the pattern proposed in 1965 (71), the data available on human IAA requirements and a series of other considerations. The 1973 FAO/WHO (72) group also recommended a scoring pattern for infants and another one for all ages beyond infancy.

The suggestion made by FAO/WHO (72) in 1973 for the use of a single reference pattern to be applied for all ages was made despite amino acid requirement data which indicated that school-age children needed some 30 percent of their protein in the form of IAA while the adult apparently needed only 15 percent or less (72). Clearly, adoption of the child pattern for purposes of amino acid scoring would underestimate the value of a protein for meeting the nutritional requirement of the adult. It was the opinion at that time (72) that since protein quality was most critical for the younger age groups, scoring patterns appropriate to these age groups should be employed for all ages. This, in practice, gave an apparent extra margin of safety to the estimation of the protein needs of older age groups and in the assessment of nutritional quality of their diets.

The 1985 FAO/WHO/UNU report (4) developed different scoring patterns for separate age groups. Additional data (75,76) for the young child were available to the 1985 group and, on the basis of this and older existing data, IAA requirement values, expressed as mg per mg body weight per day, for infants, preschool children, school children and adults were adopted. These values were then divided by the recommended safe level of protein intake (g protein per kg body weight per day) for each age group to calculate the corresponding amino acid scoring pattern (mg/g protein). For infants the amino acid composition of human milk was proposed to calculate the amino acid scoring pattern. There is no compelling reason at this time to change that as the basis for the pattern for infants. Final values proposed by FAO/WHO/UNU (4) as the scoring pattern for school-age children and adults were lower than those tabulated by the previous 1973 FAO/WHO Committee (72), even though the IAA requirement values used for developing the scoring patterns for infants, school children and adults were the same. This difference, then, was because the more recent safe levels of protein intake proposed for adults and school children had been increased over those given in the 1973 FAO/WHO report (72).

The calculation of scoring patterns for the four separate age groups (Table 7) explicitly implies that protein quality is not an unchanging attribute of protein but varies with the age of the individual consuming it. It was further concluded by FAO/WHO/UNU (4) that proteins and diets with an IAA content and pattern that effectively met the needs of young children were also adequate for older children and adults, whereas the reverse need not be true.

There have been a number of criticisms raised about the accuracy of the estimates of human IAA requirements and the scoring pattern which derive from them. Short-term balance studies in adults (77) failed to confirm the requirement values suggested by Rose et al. (78), which were the major basis for the 1985 FAO/WHO/UNU (4) adult values.

It has been pointed out (75,79) there is considerable uncertainty about the IAA requirements that had been established for school-age children (80). Problems with those studies include the excessive amount of dietary nitrogen used, the short N balance periods that did not allow for adaptation to new levels of amino acid intake, the lack of allowance for integumental and miscellaneous nitrogen losses in estimating N balance and the modification in dietary amino acid composition from one experiment to another, which influenced the outcome and interpretation of the N balance studies of Nakagawa et al. (80).

The N balance technique used for the assessment of IAA requirements has been criticized on a number of grounds (81,82). Briefly, these concerns include the inadequate criteria used in earlier studies to estimate N balance, the difficulty faced in evaluating the nutritional and health significance of a given N balance under a particular diet and experimental condition, and the complicating effects of energy intake on N balances. It has been suggested (83,84) that such problems would lead to underestimates of actual minimum physiological needs and, therefore, the relatively low requirement values proposed for the adult by the 1985 FAO/WHO/ UNU consultation (4) must be regarded with considerable circumspection.

In support of these criticisms, metabolic isotopic studies have indicated considerably higher requirement values for leucine, lysine, valine and threonine in the adult (85-90). Further, in reviewing the metabolic basis of IAA and protein requirements, it has been suggested (91,92) that the apparent age-related fall in the scoring patterns adopted by the 1985 FAO/WHO/UNU Consultation (4) primarily reflected the different dietary designs of the various original balance studies. These experimental designs would have induced different rates of oxidative losses of amino acids and, therefore, inappropriate estimates of requirements. In particular, the amino acid mixtures used in the N balance studies of Rose et al. (78) and Nakagawa et al. (80) included a disproportionate quantity of non-essential nitrogen in comparison to the composition of food proteins. Thus, various authors (83,92) now agree that there is no justification for the continued use of the scoring patterns proposed by FAO/WHO/UNU (4) for school-aged children and adults. There is, however, considerable debate as to a precise and practical alternative.

Table 7. Comparison of suggested patterns of amino acid requirements with the composition of high-quality animal proteins*

Amino acid (mg/g crude protein)	Suggested pattern of requirement				Reported composition[c]		
	Mean (range)[a]	Infant	Pre-School Child (2-5 years)[b]	School-Child (10-12 years)	Adult	Egg	Cow's milk	Beef
Histidine	26(18-36)		(19)[d]	(19)	16	22	27	34
Isoleucine	46(41-53)		28	28	13	54	47	48
Leucine	93(83-107)		66	44	19	86	95	81
Lysine	66(53-76)		58	44	16	70	78	89
Methionine + cystine	42(29-60)		25	22	17	57	33	40
Phenylalanine + tyrosine	72(68-118)		63	22	19	93	102	80
Threonine	43(40-45)		34	28	9	47	44	46
Tryptophan	17(16-17)		11	(9)	5	17	14	12
Valine	55(44-77)		35	25	13	66	64	50
Total								
including histidine	460(408-588)		339	241	127	512	504	479
minus histidine	434(390-552)		320	222	111	490	477	445

*Reproduced from FAO/WHO/UNU (4); references cited in this table are found in reference (4).
[a]Amino acid composition of human milk (16-19).
[b]Amino acid requirement/kg divided by safe level of reference protein/kg (Tables 4, 33, and 34). For adults, safe taken as 0.75 g/kg; children (10-12 years), 0.99 g/kg; children (2-5 years), 1.10 g/kg. (This age range is chosen because it coincides with the age range of the subjects from whom the amino acid data were derived. The pattern of amino acid requirements of children between 1 and 2 years may be taken as intermediate between that of infants and preschool children).
[c]Composition of cow's milk and beef (16) or egg (Lunven, P. et al., unpublished data, 1972).
[d]Values in parentheses interpolated from smoothed curves of requirement versus age.

6.2 Recommended Amino Acid Scoring Pattern

Young and colleagues (93,94) have proposed on theoretical grounds that a new amino acid scoring pattern, which is similar to that of the preschool-aged group as recommended by FAO/WHO/UNU (4), be employed for all ages except for the infant. These authors (93,94) have also provided some experimental support for the valid use of this pattern in relation to adult protein nutrition. However, the proposal made by Young et al. (93,94) and their tentative scoring pattern remain a matter of controversy (94-98).

The CCVP suggested that the scoring pattern recommended for the preschool (2-5 y) child by FAO/WHO/UNU (4) should be used for all children and adults but not for infants. The present FAO/WHO Consultation considered carefully the various arguments which had been raised and, in the light of current knowledge of the metabolic basis of indispensable amino acid needs, concluded that it was

unlikely that there was an age related fall in the IAA requirement as marked as that implied by the 1985 FAO/WHO/UNU report (4). Given the slow rate of growth of the human, it is the case that net accretion of proteins only accounts for a significant proportion of protein needs in the infant and that the maintenance component accounts for most of the requirement for all other age groups. As there is little evidence to suggest that maintenance nitrogen requirements substantially changes with age it is unlikely that IAA requirements change markedly with age.

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 decided that the scoring pattern proposed for the preschool child, which is based on various criteria of amino acid adequacy (75,76), is robust and represents the best available estimates of IAA 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 considers 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 (4) is used.

The Consultation 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.

It 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 Consultation's recommendation for an amino acid scoring pattern to evaluate protein quality.

While it is known that cystine can spare part of the requirement for methionine, FAO/WHO/UNU 1985 does not give any indication of the proportion of total sulphur amino acids which can be met by cystine. For the rat, chick and pig, the proportion is about 50%. Most animal proteins are low in cystine; in contrast, many vegetable proteins, especially the legumes, contain substantially more cystine than methionine. Thus, for animal protein diets or mixed diets containing animal protein, cystine is unlikely to contribute more than 50% of the total sulphur amino acids and scores calculated using cystine plus methionine will be appropriate. However, in certain all vegetable combinations, e.g. wheat and legumes, part of the cystine value may not be realized. Because of insufficient data on human requirements, however, the total of the two sulphur amino acids should, for the present, remain the recommended approach for computing amino acid scores.

6.3 Conclusions and Recommendations

The Consultation 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 (4) 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 Consultation.

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 programme be immediately established to assist in the determination of human amino acid needs.

7. DIGESTIBILITY METHODS

7.1 Introduction

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 fibre, 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.

7.2 In Vivo Protein Digestibility

The classic procedure for determining digestibility has been the faecal index method, an in vivo procedure in which the nitrogen excreted in the faeces is subtracted from the amount ingested and the value 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 faecal nitrogen excreted when the subject is consuming either a protein-free diet, or a diet with just enough of a highly digestible protein to prevent excessive loss of body protein. Thus, true digestibility (TD) can be calculated as:

$$TD = \frac{I - (F - Fk)}{I} \times 100$$

where I is intake nitrogen, F is faecal nitrogen, and Fk is metabolic or endogenous faecal nitrogen. Since TD measurements take into account the metabolic faecal 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 faecal 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 faeces, and the so-called metabolic losses in the faeces (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. Animal growth assays have also been used to evaluate bioavailability of amino acids. Although limited to the determination of a single amino acid at a time, the results obtained by the growth method are considered by some to be more accurate than those obtained by the balance method. The growth method is, however, more complicated and

more expensive. In a USDA organized cooperative study, bioavailabilities of some key amino acids in the same batches of foods were determined by rat growth and balance methods by different participating laboratories (1). Differences in bioavailabilities of tryptophan, lysine and methionine obtained by the two methods were 1-9, 1-13, and 3-15% respectively. These differences may be regarded as small if consideration is given to the fact that the two methods were used by different laboratories using their own analysed amino acid data.

Protein digestibility is most frequently estimated using rats. The approach is well established and the procedure has been standardized by collaborative study (2) as:

7.2.1 *In vivo rat assay for true protein digestibility*

TEST FOODS: Protein (N x 6.25), moisture, fat and total dietary fibre content of the test foods should be determined by AOAC methods. Determine nitrogen by the appropriate Kjeldahl procedure (960.52 or equivalent, AOAC, 15th edition, 1990). High moisture foods must be dried to less than 10% and fat content of high-fat foods should be lowered to 10% or less by ether extraction.

DIETS: Calculating ingredient amounts on a dry weight basis, weigh out a sufficient amount of each test food to provide 10% protein (1.6% nitrogen). Add 1% of AIN Vitamin Mix 76, 3.5% AIN Mineral Mixture 76 (Nutritional Biochemicals, Cleveland, Ohio), 0.2% choline bitartrate, 5% cellulose (only if test food is less than 5% total dietary fibre), corn oil to total 10% fat (allow for fat content of test food), and corn starch to total 100%. Mix all dry ingredients in a single batch, then add the corn oil and mix well. Use a PROTEIN FREE (2) or low protein (121) diet to estimate metabolic nitrogen; mix shall be the same as the test diets except that corn starch replaces the test food.

RAT FEEDING PROTOCOL: Male weanling rats (Sprague-Dawley) of 50-70 g shall be housed in individual cages in care rooms at 18-26° C and 40-70% relative humidity. Feed a standardized rat lab chow for an acclimation period of 2 days, then distribute rats into 2 blocks of 4 rats so mean weights of each block are within 5 g. Provide water ad libitum, but restrict diets to 15 g dry matter/ day. Feed the protein free diet and the test diet(s) for a 4-day preliminary period and a 5-day balance period (total 9 days). On each of the 5 days of the balance period, collect faeces and spilled food for each rat and carefully separate and composite in open containers (one for the faeces and one for the food). At the end of the 5-day balance period, air dry the spilled food for 3 days and deduct the weights of uneaten and spilled food from food offered to determine total food intake. Dry faeces overnight in a vacuum oven at 100° C, weigh, grind, and analyze for nitrogen.

CALCULATIONS: TD is determined as shown in 7.2. Nitrogen intake and faecal nitrogen are obtained by multiplying food intake and faecal weight by their respective nitrogen values. Metabolic nitrogen is the value obtained from the faeces of the rats fed the protein-free diet. The metabolic values, expressed in mg nitrogen/g diet consumed, are used for the other test diets, corrected for the

weight of diet consumed. For example, if metabolic nitrogen is 1.5 mg per g of protein-free diet consumed, and 50g of the test diet were consumed, then 1.5 X 50 gives the metabolic nitrogen for that test diet.

7.2.2 *Human studies*

Protein digestibility may also be obtained from human subjects using nitrogen balance studies. There has been no significant attempt to standardize procedures for protein quality evaluation studies in humans; however, guidelines have been outlined by Pellett & Young (63). Human studies would, of course, appear to be the standard for obtaining digestibility data; however safety, ethical constraints, expense, and practicality all dictate the use of animals. Comparative reviews of protein digestibility of some common foods as determined by human and rat balance methods suggested that the abilities of rats and humans to digest a variety of food proteins are similar (99,100).

7.3 In Vitro Protein Digestibility

Useful in vitro procedures based on 3 or 4 enzymes (trypsin, chymotrypsin, peptidase and bacterial protease) have been developed for predicting protein digestibility of food products (101,102). In these procedures, digestibility was estimated by measuring the fall in pH in the protein suspension caused by enzymatic digestion. Using these in vitro methods (including corrections for proteins of high buffer capacity), Pedersen and Eggum (103) estimated protein digestibility of 61 feed and food protein products. The results were reproducible with pooled standard deviations of less than 1%. In the 57 vegetable protein sources and their mixtures with animal protein sources, the positive correlations between in vitro and in vivo (rat) true protein digestibility data were significant (r=0.89 -0.90), p=0.001) (103). However the protein digestibility of egg powder, dried egg white and nonfat dry milk were underestimated by the in vitro methods. In another study, Wolzak et al (104) reported highly significant correlations between in vivo and in vitro estimates for 60 samples. However, important differences were found in processed samples which indicate more research is required for those type of samples. Pedersen and Eggum (105) introduced the use of an in vitro enzymatic pH-stat procedure in which pH was kept constant during the incubation period. Their procedure is shown below (7.3.1). With this method, protein digestibility was estimated by the amount of titrant (0.1 N NaOH) used. In general, the pH-stat procedure was more accurate than the original methods in predicting protein digestibility of food and feed products. In a further comparative study between in vivo (rats) and in vitro true protein digestibility with 17 foods, Eggum et al. (106) showed good agreements between the two measurements with the exception of two legumes (beans, chick peas) which were digested to a markedly lower degree in vivo when compared to the in vitro values. These discrepancies might partly be explained by a strong bacterial growth in the lower gut when certain legumes are consumed.

7.3.1 *In vitro assay for protein digestibility*

ENZYME PREPARATION: Prepare a solution containing all three enzymes as follows: Dissolve, in distilled water, sufficient amounts of porcine pancreatic trypsin (Type IX, Sigma 7-0134), bovine pancreatic chymotrypsin (Type II, Sigma C-4129), and porcine intestinal peptidase (Grade K, Sigma P-7520) to give, per ml, 23,100 units, 186 units and 0.052 units, respectively. Adjust pH to 8.0 at 37° C and maintain for exactly 2.0 min, then transfer to an ice bath and keep at 0° C. Prepare the 3-enzyme solution fresh daily and check activity using an aqueous suspension of sodium caseinate (1 mg N/ml distilled water) as the standard. Allow the suspension to stand at 4° C for at least 1 hr but no longer than 24 hr. Then, place 10 ml of the sodium caseinate suspension in a reaction vessel, warm to 37° C, and adjust and maintain pH at 8.0 for 5-10 min before adding 1.0 ml of the 3-enzyme solution. While stirring, record the amount of 0.1 N NaOH required to maintain pH at 7.98 for exactly 10 min and calculate true digestibility by the equation TD = 76.14 + 47.77B where B equals ml of 0.1 N NaOH added. Values for the sodium caseinate should equal 98 to 102% true digestibility.

DIGESTIBILITY ESTIMATES: Digestibilities of the test proteins are done exactly as described above, using sample amounts containing exactly 10 mg N dissolved in 10.0 ml distilled water. Sodium caseinate is used as a control to give a lab correction factor for adjusting final values as:

$$\frac{100}{\text{Sodium caseinate digestibility}} = \text{Lab Correction Factor}$$

NITROGEN ANALYSIS: Nitrogen shall be determined by Kjeldahl procedures (960.52 or equivalent, AOAC, 15th edition, 1990).

7.4 Ileal Digestibility of Protein and Amino Acids

The determination of protein and amino acid bioavailability by the balance method has been criticized because of possible microbial modifications of undigested and unabsorbed nitrogenous residues in the large intestine (107). It is well known that the pattern of nitrogen excretion is modified by the microflora present in the large intestine. This modification may cause over estimation of the digestibility of protein and availability of amino acids, especially in materials damaged by processing (108). Therefore, measuring the disappearance of amino acids from the small intestine (ileal recovery) may provide an accurate measure of their bioavailability.

However, a series of events will occur when undigested protein, both from dietary and endogenous origin (including peptides and amino acids not absorbed by the end of the small intestine) enters the large intestine. A certain proportion of the dietary protein passes through the large intestine and is excreted in faeces; the remainder is fermented by the microflora. The nitrogen will either be absorbed primarily in the form of ammonia or incorporated into microbial protein. Some of the microbial protein will be digested and the nitrogen absorbed, primarily in the form of ammonia. The remainder will be excreted in the faeces. The fate of the endogenous protein is similar to that of

dietary protein. A substantial amount of bacterial nitrogen can be found in the faeces of pigs. As was shown by Mason (109), bacterial nitrogen can amount to 62 to 76 percent of the total nitrogen in faeces. The factors that affect the microbial activity in the large intestine, including the amount of available fermentable carbohydrates are discussed by Mason (109) and Sauer and Ozimek (110).

Amino acid digestibility coefficients obtained by the faecal analysis method are, for most amino acids in most feedstuffs, higher than those obtained by the ileal analysis method. In some of the studies, net synthesis of methionine and lysine has been reported in the large intestine (111-114). Therefore, depending on the amino acid and on the feedstuff, digestibility values obtained by the faecal analysis method are overestimated (which is usually the case) or underestimated when compared to those obtained by the ileal analysis method. Lysine, the sulphur-containing amino acids, and threonine and tryptophan can be considered the more important amino acids in practical diet formulation, as these are often first-, second- or third-limiting in many food sources. Of these amino acids, cystine, threonine and tryptophan usually disappear to a significant extent in the large intestine of the pig.

In conclusion, while it is recognised that faecal true digestibility of protein has shortcomings, further methodological studies are required to resolve uncertainties, e.g. the contribution and variation of endogenous secretion at the terminal ileum, before a standardised procedure for the determination of ileal true digestibility can be recommended to replace faecal digestibility. The change to the use of ileal digestibility values, when agreed procedures and sufficient data on foods are available, can be readily implemented.

7.5 Digestibility Data

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 by Sarwar (99) and by Hopkins (115). The digestibility data discussed in those reviews were abstracted from human and/or rat balance experiments. Values for true digestibility of protein 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 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 pulses (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.

Representative digestibility values for some common foods and food mixtures are shown in Tables 8, 9, 11 and 12. 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%.

In recent cooperative studies (1,2) using the rat balance method, high true protein digestibility values of 93-100% were obtained for animal foods or food products (casein, minced beef, beef salami, skim milk, tuna, chicken frankfurters and sausage) and soy protein isolate. Intermediate digestibility values of 86-92% were obtained for chick peas, beef stew, rolled oats, whole wheat cereal, and pea protein concentrate, while low values (70-85%) were reported for different types of dry beans including pinto beans and kidney beans and lentils.

7.6 Amino Acid Digestibility

Much data has been generated using rats to compare true digestibility of protein and individual amino acids in various foods, giving evidence that differences may exist between digestibility of total protein and individual amino acids in some food products. Sarwar (99) has shown that digestibility of protein was not a good predictor of digestibility of limiting amino acids in grain legumes. For beans, peas and lentils, values for true digestibility of methionine, cystine and tryptophan were up to 43, 44, and 25% lower than those of the respective protein. However, the differences between the digestibilities of protein and most individual amino acids were less than 10% in mixtures containing animal protein sources, and low-fiber cereals and oilseed products. These data are shown in Table 9.

Using human subjects, Watts (119) reported differences in digestibility of protein and amino acids of diets containing whole egg, pork muscle or peanut butter to be not more than 5%. In another human study using beans, Blanco and Bressani (120) found only small differences in digestibility between individual amino acids and that of the protein. Except for the grain legumes, digestibility of protein was a good predictor of digestibility of individual amino acids. It therefore appears that correcting amino acid scores for true digestibility of protein is sufficient and that further correction for the bioavailability of individual amino acids is not needed for most mixed human diets.

7.7 Recommendations

1. It is recommended that studies be undertaken to compare protein digestibility values of humans and rats from identical food products.

2. Extensive evaluation of existing in vitro and in vivo methods in foods indicates that the rat balance method is the most suitable practical method for predicting protein digestibility by humans. Therefore, when human balance studies cannot be used, the standardized rat faecal-balance method of Eggum (121) or McDonough et al (2) is recommended.

Table 8. Some values (%) for digestibility of proteins in man.

	True Digestibility	
Protein source	**Mean**	**Reference**
Egg	97	4
Milk, Cheese	95	4
Meat, Fish	94	4
Maize	85	4
Rice, polished	88	4
Cottonseed	90	115
Sunflower seed, flour	90	115
Wheat, whole	86	4
Wheat, refined	96	4
Wheat flour, white	96	115
Wheat gluten	99	115
Oatmeal	86	4
Millet	79	4
Peas, mature	88	4
Peanuts	94	115
Peanut butter	95	4
Soyflour	86	4
Soy protein isolate	95	115
Beans	78	4
Corn, whole	87	115
Farina	99	115
Triticale	90	115
Corn, cereal	70	115
Wheat, cereal	77	115
Rice, cereal	75	115
Oats, cereal	72	115
Maize + beans	78	4
Maize + beans +milk	84	4
India rice diet	77	4
Indian diet + milk	87	4
Chinese mixed diet	96	4
Brazilian mixed diet	78	4
Filipino mixed diet	88	4
American mixed diet	96	4
Indian rice + beans diet	78	4

Table 9. Values (%) for the digestibility of protein and selected amino acids in various food products as determined by the rat balance method.[a]

Mixture	Protein	Lys	Met	Cys	Thr	Trp
Casein	99	100	99	100	100	100
Skim milk	95	96	92	94	95	98
Beef (roast)	100	100	100	100	100	100
Beef salami	99	99	99	100	100	100
Sausage	94	94	91	95	92	93
Egg white solids	98	97	98	97	96	97
Tuna fish	97	97	95	96	98	97
Chicken franks	96	97	97	100	95	96
Pea flour	88	92	77	84	87	82
Pea, Century (autoclaved)	83	85	62	85	78	72
Pinto bean (canned)	79	78	45	56	72	70
Lentil (autoclaved)	85	86	59	75	76	63
Fababean (autoclaved)	86	85	59	75	76	63
Soybean	90	87	82	82	84	89
Soybean protein isolate	98	98	94	94	96	98
Rapeseed protein concentrate	95	91	92	93	91	93
Peanut	96	90	85	89	89	94
Peanut meal	91	88	89	89	87	—
Peanut butter	98	96	94	100	97	99
Sunflower meal	90	87	92	91	90	—
Wheat	93	83	94	97	91	96
Rolled Oats	94	90	92	98	90	97
Rice-wheat-gluten	93	85	81	95	88	92
Wheat flour-casein	95	91	91	89	90	90
Macaroni-cheese	95	95	93	98	92	98
Potatoes-beef	86	89	83	89	83	86
Rice-soybean	90	89	77	82	84	87
Corn-pea	83	85	84	86	82	80
Corn-soybean	93	93	87	94	93	98

[a]Source, Sarwar (99).

3. Since 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), it is recommended that amino acid scores be corrected only for true digestibility of protein.

4. For new or novel products or processes, digestibility values must be determined. However, established digestibility values of well defined foods may be taken from a published data base for use in the amino acid scoring procedure, assuming all safety and toxicological criteria have been met. A data base should be established for all raw and processed products.

5. Further research is encouraged to perfect and evaluate the most promising in vitro procedures such as those of Satterlee (102) and Pedersen and Eggum, (105) for estimating protein digestibility.

8. DETERMINATION OF PROTEIN DIGESTIBILITY-CORRECTED AMINO ACID SCORE

1. Individual foods. To calculate a protein digestibility-corrected amino acid score, a test food must be analysed for proximate and amino acid compositions, and a protein digestibility value must be obtained from a data base or be determined by the rat balance method.

a. Proximate composition: Levels of total nitrogen, moisture, fat and total dietary fibre should be determined according to AOAC methods. Protein can then be calculated by using a nitrogen-to-protein conversion factor of 6.25. Foods high in moisture (such as meats) should be dried before analysis. Similarly, foods high in fat (such as meat, nuts, whole milk powder, etc.) may require a lipid extraction prior to analysis.

b. Amino acid profile: Protein hydrolysates should be prepared and analyzed for amino acids by the methods specified in Section 5.

c. Amino acid score: Amino acid ratios (mg of an essential amino acid in 1.0 g of test protein/mg of the same amino acid in 1.0 g of reference pattern for 9 essential amino acids plus tyrosine and cystine should be calculated by using the 1985 FAO/WHO/UNU (4) suggested pattern of amino acid requirements for preschool children (2-5 years). This reference pattern, shown in Table 7, contains (mg/g protein): His, 19; Ileu, 28; Leu 66; Lys, 58; Met + Cys, 25; Phe + Tyr, 63; Thr, 34; Trp, 11; and Val 35. The lowest amino acid ratio is termed amino acid score. For example, a pinto bean sample contained 30.0, 42.5, 80.4, 69.0, 21.1, 90.5, 43.7, 8.8, and 50.1 mg/g protein of His, 11e, Leu, Lys, Met + Cys, Phe + Thy, Thr, Trp, and Val, respectively. The respective amino acid (His, Ile, Leu, Lys, Met + Cys, Phe + Tyr, Thr, Trp and Val) ratios for the bean sample would be 1.58, 1.52, 1.22, 1.19, 0.84, 1.44, 1.28, 0.80, and 1.43. This would then result in an uncorrected amino acid score of 0.80 with tryptophan as the first limiting amino acid.

d. Protein digestibility: True protein digestibility should be determined using the rat balance method as standardized by McDonough et al. (2) or Eggum (121). Data on fat and total dietary fibre in the test food should be used in adjusting the formulation of test and nitrogen-free (or low nitrogen) diets. They should be equal in levels of total fat and (where possible) fibre. Cellulose should be added to the diet only when the total dietary fibre content of the test food is less than 5%. The diets should also contain approximately equal amounts of moisture and lactose (in testing high lactose foods such as milk powder).

e. Protein digestibility-corrected amino acid score of a test food should then be calculated by multiplying the lowest amino acid ratio x true protein digestibility. In this report, the score is expressed as a decimal, but it may be expressed in percentage terms. In the case of the pinto bean sample having the lowest amino acid ratio of 0.80 and a true protein digestibility of 73% [(as shown by McDonough et al (2)], the protein digestibility-corrected score would be 0.80 x 0.73 = 0.58 or 58%. Protein digestibility-corrected amino acid scores above 1.00 would be considered as 1.00 or 100%.

2. Food mixtures. For food mixtures, the full procedure for individual foods may need to be followed but when data for the amino acid composition and digestibility of the individual components are well established and only the proportions differ, the protein digestibility corrected

amino acid score can be calculated by means of a weighted average procedure. A worked example for such a calculation is shown in Table 10.

Table 10. Worked example for a mixture of wheat, chickpea and milk powder.

	Analytical Data							Quantities In Mixture				
	Weight (g)	Protein (g/100g)	Lys	SAA	Thr	Trp	Digestibility Factor	Protein (g)	Lys	TSAA	Thr	Trp
			mg/g protein					$\frac{A \times B}{100} = P$	mg PXC	PXD	PXE	PXF
	A	B	C	D	E	F	G					
Wheat	350	13	25	35	30	11	0.85	45.5	1138	1593	1365	501
Chickpea	150	22	70	25	42	13	0.80	33.0	2310	825	1386	429
Milk Powder	50	34	80	30	37	12	0.95	17.0	1360	510	629	204
TOTALS								95.5	4808	2928	3380	1134
Amino Acids mg/g protein [Total for each Amino acid/ Total protein]									50	31	35	12
Reference Scoring Pattern (Table 7) mg/g protein			58	25	34	11						
Amino Acid Score for mixture. Amino Acids/g protein divided by reference pattern.									0.86	1.24	1.03	1.09
Weighted Average Protein Digestibility Sum of [protein x factor (PXG)] divided by protein total							0.85					
Score adjusted for digestibility (0.85 x 0.86)									0.73 (or 73%)			

9. 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 x corrected score) and to replace PER in protein rating (grams protein in a Reasonable Daily Intake X PER). The amino acid score method would be the least expensive of all the suitable routine methods 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 (63). 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 labelling, 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 times 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-supplemented and proteins of the same amino acid profile, possibly due to more rapid absorption of crystalline amino acids than the protein-bound amino acids (63). 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

(63). 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 (63). Proteins completely lacking lysine (i.e., with a score of zero) can have a BV equal to 40%, due to differing needs for growth and maintenance and the capacity of an organism to adapt to low intakes of lysine (122). Similarly, proteins devoid of other essential amino acids can have BV values significantly higher than zero. Poor agreement between amino acid scores and biological estimates such as NPU can also occur at low levels of protein (123). This drawback is, however, of limited practical significance because of very few proteins or diets having extremely low levels of essential amino acids. A large discrepancy between amino acid scores and BV may also occur in the case of foods or food products containing antinutritional or toxic factors (63). In such cases, the elimination of inactivation of toxin or antinutritional factors by simple processing such as soaking/draining and/ or cooking can lead to satisfactory prediction of protein value by amino acid scores.

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 (63). 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.

Table 11. Protein digestibility-corrected amino acid scores for selected foods.

Product	Protein (Nx6.25) %	True Protein digestibility %	Amino acid score	Protein digestibility-corrected score
Casein[a]	94.7	99	1.19	1.00
Egg white[a]	87.0	100	1.19	1.00
Beef[a]	95.2	98	0.94	0.92
Pea Flour[a]	30.8	88	0.79	0.69
Pinto beans (canned)[b]	23.6	73	0.78	0.57
Pinto beans (canned)[c]	23.7	79	0.80	0.63
Pinto beans (autoclaved)[d]	19.9	80	0.77	0.62
Kidney bean (canned)[c]	18.9	81	0.84	0.68
Seafarer beans (autoclaved)[d]	23.3	84	0.84	0.70
Black beans (autoclaved)[d]	21.7	72	0.74	0.53
Fababeans (autoclaved)[d]	27.9	86	0.55	0.47
Lentils (canned)[c]	28.0	84	0.62	0.52
Lentils (autoclaved)[d]	21.9	85	0.60	0.51
Chickpeas (canned)[b]	21.2	88	0.81	0.71
Chickpeas (canned)[c]	21.4	89	0.74	0.66
Peas (Century, autoclaved)[d]	13.9	83	0.82	0.68
Peas (Trapper, autoclaved)[d]	15.7	84	0.73	0.61
Soybean protein, concentrate[e]	70.2	95	1.04	0.99
Soybean protein, isolate[e]	92.2	98	0.94	0.92
Soy assay protein[f]	93.0	95	0.97	0.92
Pea protein, concentrate[e]	57.0	92	0.79	0.73
Rapeseed protein (concentrate)[e]	68.3	95	0.98	0.93
Rapeseed protein (isolate)[e]	87.3	95	0.87	0.83
Sunflower protein (isolate)[e]	92.7	94	0.39	0.37
Wheat gluten[e]	87.0	96	0.26	0.25
Peanut meal[e]	61.2	94	0.55	0.52
Whole Wheat[a]	16.2	91	0.44	0.40
Rolled Oats[a]	18.4	91	0.63	0.57
Rice-Wheat-gluten[a]	20.3	95	0.27	0.26

[a]Data from Sarwar (99).
[b]Data from Eggum et al (106).
[c]Data from Sarwar et al (117).
[d]Data from Sarwar and Peace (116).
[e]Data from Sarwar et al. (125).
[f]Data from Sarwar (118).

10. SOME APPLICATIONS OF PROTEIN DIGESTIBILITY-CORRECTED AMINO ACID SCORES

10.1. Foods and Food Products

Table 11 provides base data on digestibility of some protein sources, and illustrates the affect of digestibility on amino acid scores. The scores for various types of beans, lentils and peas ranged from 0.47 to 0.71. These products were first limiting in sulphur amino acids and/or tryptophan for human nutrition. All contained less than 30% total protein. Digestibilities of the legumes ranged from a low 72% for black beans to a medium 89% for chick peas. The soybean products all had high digestibilities (90-98%) and high corrected amino acid scores (0.92-0.99). The protein digestibility-corrected amino acid scores for pea proteins were 0.61-0.78, based on tryptophan and/or sulphur amino acids as the first limiting amino acid(s). The higher score for pea protein concentrate than for whole peas was due to improved protein digestibility.

The rapeseed protein products had fairly high protein digestibility-corrected amino acid scores (0.83-0.93), with lysine being the first limiting amino acid. Wheat gluten and sunflower protein isolate were severely limiting in lysine and had low protein digestibility-corrected scores of 0.25 and 0.37, respectively.

Breakfast cereals such as rice-wheat gluten, whole wheat and rolled oats were highly digestible, but low lysine levels resulted in low protein digestibility-corrected amino acid scores of 0.26, 0.40 and 0.57, respectively. A sample of peanut meal had a protein digestibility-corrected score of only 0.52, and was co-limiting in several essential amino acids such as methionine + cystine, lysine, threonine and/or tryptophan.

Animal protein products such as egg white, casein, and ground beef (Table 11), and beef salami, skim milk powder, tuna, and chicken frankfurters (not shown) were all highly digestible (94-100%), and had corrected amino acid scores of 0.92-1.00 (99). A sample of pork sausage had, however, a relatively low protein digestibility-corrected score of 0.63 due to deficiency in tryptophan.

The low protein quality of a vegetable protein source can be improved by the addition of supplementary protein or the limiting amino acid, and by protein complementation. The addition of amino acids to increase protein quality of a protein source should only be considered when protein supplementation or complementation have proved impracticable (125) since benefits from the addition of amino acids have not been demonstrated consistently in humans (126). Furthermore, an excess of a supplementary amino acid such as synthetic methionine may have a deleterious effect on infants and children (125).

Data on the protein digestibility-corrected amino acid scores of some protein mixtures having supplementary and/or complementary effects are given in Table 12. The protein digestibility-corrected amino acid score of whole wheat flour of 0.41 was improved to 0.67-0.91 by the addition

of rapeseed protein concentrate, soy protein, egg white, pea flour, beef or casein. Similarly, the addition of ground beef gave considerable improvement in the protein digestibility-corrected amino acid scores of wheat gluten (0.25 vs. 0.77), sunflower protein isolate (0.37 vs. 0.84), pea protein concentrate (0.73 vs. 0.80) and peanut meal (0.52 vs. 0.76) (Tables 11 and 12).

Table 12. Protein digestibility-corrected amino acid scores for some protein mixtures.[a]

Mixture[b] (50:50 protein basis)	True Protein digestibility %	Amino acid score	Protein digestibility-corrected score
Wheat flour (WW)	90	0.46	0.41
WW + beef	93	0.91	0.85
WW + egg white	95	0.83	0.79
WW + casein	95	0.96	0.91
WW + rapeseed concentrate	93	0.72	0.67
WW + pea flour	92	0.89	0.82
WW + soy protein	92	0.78	0.72
Beef + rapeseed concentrate	95	1.12	1.00
Beef + rapeseed isolate	96	1.12	1.00
Beef + soybean concentrate	96	1.17	1.00
Beef + soybean isolate	98	1.07	1.00
Beef + peanut meal	95	0.80	0.76
Beef + pea concentrate	95	0.84	0.80
Beef + sunflower isolate	95	0.88	0.84
Beef + wheat gluten	95	0.81	0.77

[a]Source, Sarwar (99).
[b]Data for individual products are in Table 11.

10.2 Infant Formulas

The scoring pattern based on amino acid composition of breast milk should be used in calculating amino acid scores for infants younger than 1 year. The requirement pattern for infants (4) has been used in calculating amino acid scores of commercially available infant formulas in Canada and the United States (127). From examination of these amino acid scores, the authors suggested that the protein in milk- and soy-based infant formulas could be limiting in amino acids essential for growth of infants. In practice, the amino acid profiles of the infant formulas are, however, adequately compensated for by the higher level of protein in infant formulas compared to human milk, resulting in no evidence of amino acid deficiencies in clinical studies. Assessment of amino acid adequacy of infant formulas (a sole source of nutrition) should, therefore, be based on a method that takes into account both quality and quantity of protein. One such method, termed "amino acid rating" has been developed (127).

Amino acid profiles and protein digestibility (by the rat balance method) for various forms (powder, ready-to-use, liquid concentrate, etc.) of cow's milk- and soy-based infant formulas obtained from four manufacturers have been determined (127, 128). The product of amino acid score and total protein (g/100 kcal) was termed "amino acid rating." Amino acid scores for the milk- and soy-based formulas ranged from 0.59 to 0.90 and from 0.59 to 0.81, respectively, due to deficiencies in sulphur amino acids and/or tryptophan. Protein digestibility values in milk- and soy-based formulas ranged from 87 to 97% and from 92 to 95%, respectively. When corrected for protein digestibility, the relative amino acid ratings for all infant formulae, except the liquid-concentrate forms of the milk-based formulae (77-98%), were above 100%.

The protein quality and adequacy data for the milk-based formulas suggested that liquid concentrates may be inferior to powders prepared by the same manufacturers, possibly due to more heat treatment involved in their preparation (128). These observations support the need to investigate the effects of processing used in the preparation of various forms of milk-based formulas on their amino acid bioavailability and protein quality in infants. In these studies, the FAO/WHO/UNU (4) requirement pattern, based on amino acid composition of human milk, was used in calculating amino acid scores. However, according to European Community (129) compositional requirements for infant formulas, human milk contains significantly lower levels of methionine + cystine (2.9 g/100 g protein) than those (4.2 g/100 g protein) reported by 1985 FAO/WHO/UNU (4), although the difference in contents of other essential amino acids of human milk were small. A more recent investigation on amino acid composition of human milk (130) supports the high values for methionine + cystine, as reported by FAO/WHO/UNU (4). Further data on amino acid profile of human milk using improved and standardized methods of analysis are required to confirm the requirement pattern for calculating scores of infant formula.

10.3 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 therefore recommends that the procedure be adopted as the preferred method of measuring protein values in reference to human nutrition.

11. SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

1. The Consultation 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. It 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. It 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. The Consultation 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. The Consultation agreed that the rat balance method is the most suitable practical method for predicting protein digestibility by humans.

6. Based on the above conclusions, the Consultation 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 further recommended:

- further research must be carried out to confirm the currently accepted values of protein and amino acid requirements of infants and pre school-aged children and to define the amino acid requirements of school-aged or adolescent children and of adults;

- that FAO/WHO coordinate international research programmes 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 (68) with reliable amino acid data and commission new analyses of foods where there are insufficient reliable data.

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Annex 1

JOINT FAO/WHO EXPERT CONSULTATION ON PROTEIN QUALITY EVALUATION BY AMINO ACID SCORING PROCEDURE

BETHESDA, MARYLAND, USA

4-8 DECEMBER, 1989

AGENDA

1. Opening of the Meeting

2. Adoption of the Agenda

3. Importance of Protein Quality Evaluation
   - Meeting regulatory needs
   - Economic impact

4. Techniques for Evaluating Protein Quality: A Summary
   - Clinical methods
   - Biochemical parameters
   - Microbiological methods
   - Animal bioassays
   - Chemical methods

5. Use of Amino Acid Composition Data to Predict Protein Quality
   - Background
   - Human amino acid requirements
   - Amino acid assay methods
   - Data from human studies
   - Digestibility/availability considerations
   - Scoring procedures

6. Adoption of Framework for Discussions and Report

7. Recommendations

8. Adoption of Report

JOINT FAO/WHO EXPERT CONSULTATION ON PROTEIN QUALITY EVALUATION WASHINGTON, D.C.

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GLOSSARY OF TERMS USED IN PROTEIN QUALITY EVALUATION

Annex 3

Many terms and ratios have been used in relation to protein quality. This glossary is an attempt to define, and hence standardize, the terminology used in protein quality evaluation. The presence of terms does not necessarily imply approval by the consultation but merely recognition that the term has been proposed and is in use. New terms that are equivalent to others already in use have been so identified. It would often be more accurate for the words "claimed to indicate" to precede some of the definitions because it was not always agreed that the indices, in fact, measured the parameters they were purported to measure. Indeed, criticism of many of the terms defined in this glossary will have been found throughout the text.

The word "protein has a common usage, and the reader is referred to the more precise meanings of crude protein, reference protein, protein calories, and protein-energy ratio. In most of the methods described for the determination of nutritive value, nitrogen is used as an index of protein, and thus the definitions relate strictly to the nutritive value of the nitrogen of foods. Where possible, each term has been defined in a general sense, and the responsibility of more precise meaning is left to the user. Thus, in general, "net protein utilization" is defined as the fraction of food nitrogen intake that is retained, but the conditions under which a particular measurement has been made should be stated, e.g., the age or weight, sex, and species of the animal used, its energy intake, the composition of the diet fed (especially the protein and energy contents), details of the experiment, including the period of measurement, the environmental temperature, and the previous nutrition of the animals. Other factors may relate to cage size and whether the animals are housed singly or together.

The following abbreviations have been used:

A = absorbed nitrogen = $I - (F - F_k)$
B = body nitrogen
B_k = body nitrogen at zero nitrogen intake
B_o = body nitrogen at zero time
F = faecal nitrogen
F_k = metabolic nitrogen (endogenous faecal)
I = intake nitrogen
S = integumental and miscellaneous nitrogen
S_k = obligatory integumental and miscellaneous nitrogen
U = urinary nitrogen
U_k = endogenous urinary nitrogen

B and B_k are measured at the end of the test period in animals fed the test diet and non-protein diet, respectively. B_o is measured on a control group of animals at the beginning of the experimental period.

Amino acid rating
Amino acid score X total protein (g/100 kcal)

Amino acid score

$$\frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in 1 g of reference protein}}$$

In practice, equivalent to "chemical score" and "protein score", although "chemical score" as originally defined was relative to the amino acid composition of egg protein. Expressed either as a ratio to unity (recommended), or on a percentage scale. Score usually calculated from "first limiting amino acid" in the test protein, but may sometimes be used for other than the limiting amino acid.

Available amino acids
Amino acids in the food of an animal that are available for protein anabolism. These may be measured directly by bioassay, or indirectly by special chemical or microbiological methods.

Available lysine value (ALV)
A chemical determination of lysine in a form that will react with fluorodinitrobenzene (FDNB). More recently termed "FDNB-available" or "FDNB-reactive lysine" to avoid confusion with bioassays for available amino acids (q.v.).

Biological value (BV)
The proportion of absorbed nitrogen that is retained for maintenance and/or growth, i.e., B/A, or

$$\frac{I - (F - F_k) - (U - U_k)}{I - (F - F_k)}$$

It may also include sweat nitrogen losses and would then be defined as

$$\frac{I - (F - F_k) - (U - U_k) - (S - S_k)}{I - (F - F_k)}$$

If the correction of metabolic and endogenous losses is not made, the value is termed apparent biological value, i.e.,

$$\frac{I - F - U}{I - F}$$

May also be defined in terms of carcass nitrogen, in which case the definition for BV and apparent BV become

$$BV = \frac{\Delta B}{A} = \frac{B - B_k}{I - (F - F_k)}$$

$$\text{Apparent BV} = \frac{B - B_o}{I - F}$$

Expressed either as a ratio to unity (recommended) or on a percentage scale.

Association of Official Analytical Chemists (AOAC)
An international association (based in North America) of analytical chemists who can approve analytical procedures. When so approved, such procedures are often acceptable internationally.

Chemical score
The content of each essential amino acid in a food protein is expressed as a percentage of the content of the same amino acid in the same quantity of a protein (real or hypothetical) selected as a standard. The original standard used was egg protein. The amino acid showing the lowest percentage is called the "limiting amino acid" and this percentage is the chemical score. The concept is applicable to both available amino acid and total amino acid data. Score is thus dependent on the standard chosen. It is frequently used interchangeably with "amino acid score" and "protein score." May now also be expressed as a fraction.

Crude protein
Nitrogen content multiplied by the conventional factor 6.25: crude protein = N x 6.25.

Digestibility
The proportion of food nitrogen that is absorbed:

$$\frac{A}{I} = \frac{I - (F - F_k)}{I} = \text{true digestibility}$$

This value is often termed "true digestibility of nitrogen." If the correction for metabolic losses in faeces is not made, the value is termed "apparent digestibility":

$$\frac{I - F}{I} = \text{apparent digestibility}$$

Essential amino acid (EAA)
An amino acid that cannot be synthesized from materials normally present in the diet at a rate commensurate with normal bodily needs. Also called "indispensable amino acid" by some investigators.

Gas liquid chromatography (GLC)
Commercial equipment for the rapid resolution of mixtures in the vapor phase. For the analysis of amino acids, the preparation of volatile derivatives is necessary.

High performance liquid chromatography (HPLC)
Commercial equipment for liquid chromatography often using high pressure to give rapid resolution. Can use both pre- and post-column derivatization in the analysis of amino acids.

Ion exchange chromatography (IEC)
A procedure using ion exchange resins and post-column derivatization for the analysis of amino acids.

Indispensable amino acid
See "Essential amino acid."

Limiting amino acid (LAA)
The essential amino acid of a protein present in the lowest proportion as compared to the same quantity of another protein (real or hypothetical) selected as a standard. The apparent limiting amino acid in a protein is thus dependent on the standard chosen. The true limiting amino acid in a protein is, however, the amino acid limiting growth in a biological experiment. See "Chemical score," Amino acid score," and "Protein score."

Net protein ratio (NPR)
The weight gain of a test animal plus weight loss of a control animal per gram of protein consumed. Thus:

$$\text{NPR} = \frac{\text{weight gain of test animal} + \text{average weight loss of animals fed basal (non-protein) diet}}{\text{protein (N x 6.25) consumed by test animal}}$$

Both 10- and 14-day growth periods have been recommended. An improvement on protein efficiency ratio (q.v.) in that an allowance for maintenance is made by use of a non-protein control group. Similar in concept to net protein utilization (q.v.) but calculated from body weight rather than body nitrogen. As defined above, the ratio is not on a percentage or unity scale.

Net protein utilization (NPU)
The proportion of nitrogen intake that is retained, i.e., the product of biological value (q.v.) and digestibility (q.v.).

$$\text{NPU} = \text{BV} \times \text{D} = \frac{\triangle B}{A} \times \frac{A}{I} = \frac{\triangle B}{I} = \frac{I - (F - F_k) - (U - U_k)}{I}$$

May also be defined in terms of carcass nitrogen when

$$\frac{\triangle B}{I} = \frac{B - B_k}{I}$$

In this case, digestibility is included in the index and cannot be expressed separately unless faecal analysis is performed.

If the measurement of NPU is made under standard conditions, with the protein intake at 10 per cent (100 g per kg diet) or below, the value is termed standardized. If a food or diet is fed as it is consumed without dilution or addition, it is termed NPU operative (NPU_{op}). Similar to net protein ratio (q.v.) if body weight is used to calculate body N.

If the correction for endogenous losses is not made, the value is termed apparent NPU, i.e.,

$$\text{Apparent NPU} = \frac{I - F - U}{I} \quad \text{or} \quad \frac{B - B_o}{I}$$

Net protein value (NPV)
A term used to compare protein concentrates. The product of the percentage of crude protein and NPU measured with diets containing 10 per cent protein, i.e., crude protein % x NPU_{10}. Similar, but not equivalent, to utilizable protein (q.v.).

Nitrogen balance
Apparent nitrogen retention (see "Nitrogen retention"), i.e., I - F - U.

Nitrogen-balance index (NBI)
The slope of the line relating nitrogen balance to absorbed nitrogen. In many circumstances this is equivalent to biological value (q.v.). If nitrogen intake is used in place of absorbed nitrogen, the values are equivalent to net protein utilization (q.v.).

Nitrogen conversion factors
Various factors have been proposed for the conversion of the nitrogen content of different foods to protein content; they may range from 5.18 for almonds to 6.38 for milk. In this publication protein is always N x 6.25.

Nitrogen growth index (NGI)
The slope of the line, using linear regression analysis, relating growth to nitrogen intake. In some circumstances, equivalent to net protein ratio.

Non-specific nitrogen
Nitrogen that is metabolically available but that leads to minimal toxicity at the levels used.

Precision
The ability of an estimate to discriminate quality among proteins is a function of both how different the estimates are and also the random error or coefficient of variation of the estimate.

Proportionality
An estimate should be proportional. A material with half the potency of another should yield estimates that are half the value.

Protein
See the introductory paragraphs to this Glossary. See also "Nitrogen-conversion factors," "Crude protein," "Protein calories," and "Protein calories percent."

Protein calories
The metabolizable energy (kcal) of crude protein, i.e., N x 6.25 x 4, or N x 25.

Protein calories percent (PCal%)
Protein calories (q.v.) expressed as percentage of total metabolizable energy (kcal).

Protein efficiency ratio (PER)
Weight gain per weight of protein eaten. Values are usually measured using rats. Originally measured at different levels of protein and the maximum value quoted; later conventionally fed at 10 percent protein; standardized procedure uses diets containing 9.09 percent protein.

Protein requirement
Used loosely to describe the overall protein needs of population groups.
Has been defined for each subgroup of the population, as the sum of obligatory nitrogen losses together with the special nitrogen needs (where applicable) of growth, pregnancy, and lactation. These requirement values (mg N/kg/day) are then adjusted upwards by factors to allow first for the inefficiency of nitrogen utilization and then for individual variability. After multiplication by body weight and conversion to protein (N x 6.25), followed by further upward adjustment to allow for protein quality, these values become the safe practical allowance (SPA) or recommended dietary allowance (RDA) for protein (g/day) for specific population groups. May be defined by other criteria for the young child.

Protein score
Measures the extent to which a food or food combination supplies the limiting amino acid as compared to the provisional pattern. See "Chemical score" and "Amino acid score."

Reference pattern
The pattern of amino acids in a reference protein. Also used in a less rigorous manner to mean a pattern of amino acids used for reference.

Reference protein
A hypothetical protein of high biological value containing a specified pattern of amino acids. (Hypothetical because it is assumed to have the same quality at any dietary level; this is an invalid assumption for food proteins.) Used for stating protein requirements. Similar, but not equivalent, to net protein value and utilizable protein (q.v.).

Relative
The term is used preceding a defined index when that index is expressed in relation to the value obtained at the same time, under the same conditions, with a standard protein taken as unity. May also be expressed in terms of percentage.

Relative net protein ratio (RNPR)
NPR of a test protein expressed as a fraction of that obtained with a standard high-quality protein, taken as unity (recommended) or as a percentage.

Relative protein value (RPV)
The slope of the straight portion of the line relating growth response to nitrogen intake, i.e., protein value (PV) (q.v.) expressed on a scale relative to 1.00 for a standard high-quality protein. This was originally lactalbumin. The slope should not include the zero (non-protein) data. Growth response may be expressed as live weight, body water, or body nitrogen.

Repeatability
The variation of an analytical procedure when replicated under the same conditions within one laboratory. It is calculated from the between replicate mean square and expressed either in absolute units as the standard deviation or in percentage units as coefficient of variation.

Reproducibility
The variation arising from different operators, apparatus and laboratories. It is expressed either in absolute units as the standard deviation or in percentage units as coefficient of variation. The value indicates the variation between a single analysis carried out in one laboratory and a single analysis carried out in a different laboratory on the same sample by nominally the same method.

Slope ratio assays
A general term to describe assays of the dose-response type where the slope of the response to the test substance is expressed as a ratio of the slope of the response to the standard substance. For most assays, dose (X) is protein or nitrogen intake, while response (Y) would be body weight, body nitrogen, body water, N balance, feed conversion efficiency or other appropriate response parameters. The straight-line portion of the relationship is used for the calculation of slope and intercept by linear regression analysis.

Standard protein
A high-quality protein used in a biological assay procedure as a reference. Should be determined at the same time and under the same conditions as the assay procedure being used. Not identical to reference protein.

Sulphur amino acids (SAA)
The total of methionine and cystine used for scoring purposes. Units are as for other amino acid data, i.e., mg/g N or mg/16 g N. Cystine is not an essential amino acid but can be synthesized from methionine. Cystine in a diet can thus "spare" methionine, and the total of the two has been found more satisfactory for scoring purposes than methionine alone. Sometimes called "total sulphur amino acids."

Total sulphur amino acids (total SAA)
See "Sulphur amino acids."

Utilizable protein
The potential maximum amount of protein present that can be utilized. A multiple of protein content (g/100 g or g/kg) and a quality index (as a fraction).

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