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Nutritional Standards and Methods of Evaluation for Food Legume Breeders

Prepared by the International Working Group on Nutritional Standards and Methods of Evaluation for Food Legume Breeders

J.H. Hulse, K.O. Rachie, and L.W. Billingsley

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Calculation of N:P Factor

1. The amino acid (AA) analyzer should be programed to yield the data for grams AA nitrogen (N) per 100 g of total N, or the equivalent = I.

2. Derive the corresponding figures for grams AA residues per 100 g protein. In proteins the AAs are linked by peptide bonds. The elements of water are added to each molecule during hydrolysis, so that the recovery of AAs should be greater than 100%. To get a true picture of the relationship of N to AAs, the AA figures from hydrolyzates must be corrected to the original residue form in which they exist in proteins.

Divide the figures from series I by an individual factor, K_{AA} for each AA. The K_{AA} constants depend on the original N:P factor used in reporting the "protein" content of the sample, and the molecular weight and nitrogen content of the individual AAs.

Grams AA residues/100 g protein = II.

The K_{AA} constants for all common AAs are listed in Appendix Table 1 for N × 5.7 and N × 6.25.

3. Total series II and divide by (100 ÷ % protein in original sample), to arrive at % AA residues in original sample = III.

4. Divide III by % N in original sample to derive the ratio of total N : total AA residues. This is the true N:P factor. A typical calculation follows in Appendix Table 1.

5. Ensure that all AA, "protein" and total N results are reported on the same moisture basis (preferably moisture-free) to avoid serious errors in computation of N:P factor.

Biological Assays for Protein Quality

J.M. McLaughlan

It has been known for more than a century that proteins differ in their nutritional value for animals. Gelatin, in particular, was shown to be much inferior to most other proteins. The concept of "biological value" and the first real protein methodology for nutritional evaluation was proposed by Thomas in 1909. Although the method, applied and modified by Mitchell (1), is theoretically sound, it is too laborious and time consuming for ordinary use. In 1919, Osborne et al. (2) introduced a simple rat growth assay called protein efficiency ratio (PER); this procedure, with some modification, is now probably the most widely used method for evaluating protein quality. PER is influenced by several factors, but the major criticism of the method is that individual PER values are not proportional; a PER of 2.0 is not twice as good as a PER of 1.0. This problem arises because PER does not make allowance for protein utilized for maintenance purposes.

Bender and Miller (3, 4) introduced an assay for net protein utilization (NPU) referred to as the carcass analysis method, which does allow for protein required for maintenance. NPU is widely used, but it is also laborious because it is necessary to measure the nitrogen content of rat carcasses. Bender and Doell (5) later proposed a simple modification of NPU in which body weight rather than body nitrogen was measured; the method was called net protein ratio (NPR). This is the same as PER but adding the weight *loss* of the nonprotein group to the weight gain allows calculation of values from poor-quality proteins that do not support growth. Several groups of workers have shown that changes in body weight accurately reflect changes in body nitrogen in short-term (i.e., 10–14 days) tests (6, 7). Body weight can be determined readily and with less error than body nitrogen; therefore, the use of body weight instead of body nitrogen reduced the variability of assays.

Until recently, PER was the method of choice in North America, whereas NPU was more commonly used in Europe. In 1965, Hegsted and Chang (8, 9) proposed a multidose slope-ratio assay. This was a modification of the nitrogen balance index devised by Allison and Anderson (10), which has been used extensively by Bressani and co-workers (11). Hegsted and Chang (8, 9) claimed that the slope ratio had the characteristics of a good bioassay — provided that the body weight response was linear over the range of protein levels involved in the estimation of the slope. A reference standard protein was included in each assay and the slopes of the response lines for reference and test proteins were expressed as ratios. For a valid slope-ratio assay the response

lines should be linear and meet at a common point on the Y-axis, which should be the weight loss of the rats fed a nonprotein diet. Experience with the slope-ratio method has shown that this is not usually true, particularly for lysine-deficient proteins (12, 13). Consideration is being given to using the true slopes of the response lines and not including the group of rats fed the nonprotein diet in the calculation of the slopes (14). This modified slope-ratio assay is called relative protein value (RPV).

The relations between PER, NPR, and multi-dose slope-ratio assays are illustrated in Fig. 1. Weight changes with four doses of protein are plotted. The response line "d" shows the ideal relation. PER is a one-dose assay; NPR is a two-dose assay; and slope ratio is multi-dose.

$$\text{PER} = \frac{\text{weight gain of test group (g)}}{\text{protein consumed (g)}}$$

$$\text{PER at weight gain of 52.5 g} = \frac{52.5}{10.5} = 5.0$$

$$\text{PER at weight gain of 20 g} = \frac{20}{5.25} = 3.81$$

$$\text{NPR} = \frac{\text{weight gain of test group (g)} + \text{weight loss of nonprotein group (g)}}{\text{protein consumed (g)}}$$

$$\text{NPR at weight gain of 52.5 g} = \frac{a+b}{c} = \frac{52.5+12.5}{10.5} = 6.19$$

$$\text{NPR at weight gain of 20 g} = \frac{20+12.5}{5.25} = 6.19$$

The slope of response line "d" as used in the slope-ratio assays

$$= \frac{a+b}{c} = \frac{52.5+12.5}{10.5} = 6.19$$

The most obvious point is that the NPR value is the slope of the response line. Consequently NPR and slope-ratio assay give identical results if the assay is perfect (i.e., all doses falling on the straight-line response). With PER there is no relation between PERs at different protein levels. In Fig. 1 the PER is zero with a protein intake of 2.0 g.

PER is a one-dose assay carried out at 9–10% protein level in the diet. NPR is a two-dose assay (8–10% and zero protein). The slope of the response in the multidose assay is influenced by the two terminal doses. It is not surprising, therefore, that there is generally good agreement among protein quality methods. Nevertheless, there are significant differences between methods for low quality proteins — this is illustrated in Table 1. Each protein quality method is expressed on a scale of 1 to 100, and lactalbumin is arbitrarily set at 100. The customary protein quality assay diet contains 10% lipid. When the diet contained 20% lipid the rats ate less food; the lower food intake affected the PER assay to a greater extent than the other two methods. A poor protein such as pea had a low PER that increased fourfold by supplementation with methionine; the other two methods merely showed a doubling of protein quality. Lysine-deficient protein such as wheat gluten had a very low value by PER; NPR gave a relatively high value and the result by the RPV assay was intermediate. The last three samples, bread, beans, and bread plus beans, illustrate supplementary effects between proteins. Bread and beans individually had low protein quality values, but the mixture of the two proteins had a much better value than either alone. Bread is deficient in lysine but has a relative abundance of methionine plus cystine; the reverse is true for beans. Supplementary effects can be readily predicted using amino acid score (chemical score).

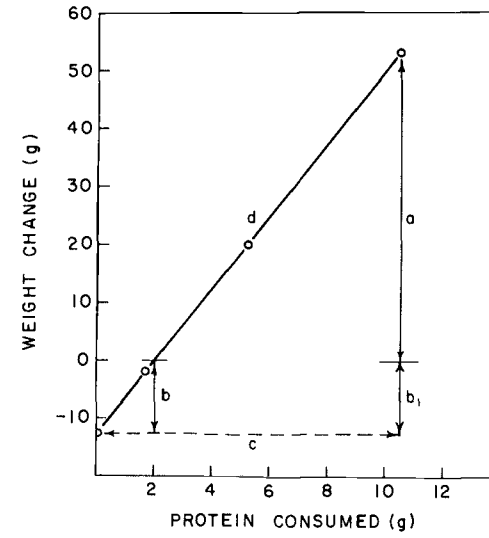

Fig. 1. Theoretical "ideal" relation between weight change of rats fed four levels of dietary protein and protein consumed.

The use of several different methods for estimating protein quality means that there is no

unanimity as to which is the most suitable method for routine biological evaluation of food proteins. Each of the methods discussed has serious shortcomings. Hegsted and co-workers (8, 9) have critized one- and two-dose assays (BV,[1] PER, NPU, and NPR) and have pointed out the theoretical advantages of a multidose assay, but there are problems with lysine-deficient proteins (12, 13) for the slope-ratio assay.

The question arises, why does the biological evaluation of protein quality with rats present such problems? The difficulties may relate to what we mean by "protein quality." Some workers consider protein quality to be a fixed entity, an inherent property of the protein, but others consider it to be a variable characteristic. It is generally agreed that protein quality for the rat depends largely, but not entirely, on the concentration of the limiting amino acid and the rat's requirement for that amino acid. As the rat's requirements for specific amino acids probably differ for maintenance and growth purposes (12, 14), protein quality may differ at different levels of protein intake (i.e., levels required for maintenance and for growth). In view of the factors involved in protein quality (different limiting amino acids in different proteins and the effect of different protein levels), it is probably impossible to devise a fully satisfactory assay for protein quality. Nevertheless, even PER is a useful measure, particularly for good-quality proteins. A recent collaborative assay indicated that both NPR and slope-ratio are superior to PER.

It seems likely that either the NPR or the RPV method will replace PER as the most widely applied method for estimating protein quality (15). The RPV assay producing ideal data yields exactly the same value as NPR. However, the NPR method gives higher values than the RPV method for lysine-deficient proteins. At present, it is not possible to decide which result is correct. One problem with the slope assay is the need for larger amounts of test protein due to multiple doses, and this may limit its usefulness in plant breeding programs.

Amino acid score (chemical score), which is based on the amino acid composition of the protein, is a rapid, useful method for estimating protein quality. This approach was originally proposed by Block and Mitchell (16) who used the essential amino acid pattern of whole egg as a reference. Egg protein was presumed to have an ideal amino acid pattern and it gave the highest protein quality measures in both man and the growing rat. Each of the essential amino acids in the test protein was expressed as a percentage of the amount of that amino acid in egg. Chemical score of a test protein was the value for the amino acid in greatest deficit relative to egg protein. Chemical score indicated both the limiting amino acid and the relative nutritive value of the protein. Although several studies have demonstrated the value of chemical score other studies have shown that the sulfur-containing amino acids (methionine and cystine) are too high in egg for a good reference protein (17).

Table 1. Comparison of values for several proteins by three methods (lactalbumin = 100).

Protein source	PER	NPR	RPV
Lactalbumin	100	100	100
Lactalbumin (20% lipid)	82	94	95
Pea	19	47	36
Pea + methionine 1	58	69	68
Pea + methionine 2	85	86	83
Soya flour	68	73	74
Wheat gluten	5	37	23
Bread	17	38	—
Beans	20	45	—
Bread + beans	54	64	—

Several other amino acid scoring patterns have been suggested, but the latest and probably most appropriate pattern is based on human amino acid requirements (18). Despite considerable differences in the amino acid content of the various scoring patterns, cereals are always deficient in lysine, and legumes are limiting in methionine plus cystine (a few may be equally limiting in tryptophan). Several studies have shown a high degree of correlation between lysine content and PER of a wide variety of cereals.

In using amino acid score, the assumption is made that amino acids are fully available — this isn't always true. Digestibility, particularly for beans is a real problem (19). Amino acid score gives an excellent picture of the potential nutritive value of a protein — but the actual value may be lower due to incomplete availability of amino acids. If amino acids were completely available there wouldn't be any need for a bioassay for protein quality.

[1]BV, biological value.

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Criteria for Cooking Quality and Acceptability of Cowpeas

Florence E. Dovlo

Because of limitations in the amino acid composition of proteins in leguminous grains, such grains have poor nutritional quality. For this reason, there is currently a growing concern for improvement of the quality of protein in these relatively inexpensive food legumes to increase their nutritional contribution to diets of low-income groups in particular, and also to make cowpea meals suitable for infant feeding.

Legume breeders are urged to include improvement of the amino acid profiles of legume proteins in their breeding programs, and to eliminate certain undesirable characteristics of food legumes, as well as identifying high-yield genotypes that are also weevil-resistant. It is equally important for breeders to take into account the cooking quality and consumer acceptance of the new cowpea cultivars.

Cowpeas (*Vigna unguiculata*) are among the most widely used of the food legumes. Cowpeas are of different sizes, shapes, and colour, and are used in a great variety of ways (1). In addition to their visual characteristics, cowpeas have intrinsic differences in their cooking quality, texture, and flavour. Consumer studies show that consumers have particular preferences for various uses of the different types of cowpea. It is therefore essential that legume breeders be cognizant of these preferences (2). For consumer acceptance it is important that the type of cowpea be fast-cooking and capable of doubling in quantity. Ability of the grains to bind is another desired quality for certain types of dishes. Taste and flavour are important factors for consumer acceptance.

For plain cooking, and for combinations with cereals, the brown type of cowpea is preferred and generally used to avoid monotony in the colour of the dish. The bright maroon-red cowpeas are usually preferred for stew, with the grains remaining firm after cooking.

Cowpeas are also processed into paste or flour and used in making certain cowpea dishes that are fried or steamed. In this process, the grains are first dehulled, then ground to make the paste, or alternatively, dried and ground into flour. For this operation, ease of soaking and dehulling are essential. The cream-coloured cowpea has been found easiest to dehull (2).

For some processed cowpea dishes, the paste or flour is whipped before use. In this usage, desirable characteristics of the flour or paste are