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

This publication was prepared by the International Development Research Centre in cooperation with the International Union of Food Science and Technology, the International Union of Nutrition Sciences, the International Centre of Tropical Agriculture (CIAT), the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and the International Centre for Agricultural Research in Dry Areas (ICARDA).

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Calculation of the Nitrogen-to-Protein Conversion Factor

R. Tkachuk

The crude protein content of plant and other food materials is estimated by multiplying its total nitrogen (N) content by some factor, hence the term nitrogen-to-protein (or N:P) factor. The factor of 5.7, widely used for wheat, has its origins in the painstaking work of Osborne and Voorhees (1), who found that the average nitrogen content of the protein fractions they isolated was 17.54%. It was subsequently concluded that by determining the nitrogen content of any sample of wheat and multiplying the result by $100 \div 17.54$ (5.70), a good working estimate of the protein content would be realized. The principle has been extended to other commodities, as various workers have isolated and analyzed the proteins for nitrogen. Some of the more commonly used N:P factors are: wheat and wheaten products, 5.7; rice and rice products, 5.95; all other cereals, legumes, oilseeds, and forages, 6.25; and milk and dairy products, 6.38.

Proteins and peptides are polymers of amino acids (AA). The availability of accurate amino

acid analysis now makes it possible to calculate the protein content of various materials more accurately. Dividing the protein content calculated from the AA composition by the nitrogen content of the sample leads to an accurate value for the N-to-P factor. When such calculations were carried out for some cereals and oilseeds (2, 4), N-to-P factors obtained were equal approximately to 5.5-5.7. The procedure of using 6.25 as a factor for all oilseeds, pulses, forages, and cereals (other than wheat and rice) is of doubtful validity. This factor was derived largely as a result of the analysis of proteins isolated from animal sources. Analytical studies made on a wide diversity of cereals and oilseeds have failed to identify any materials with a factor as high as 6.25 (2, 5).

Aside from the commercial aspect, the chief significance of the N:P factor concerns the nutritionist, who endeavours to formulate diets with a satisfactory balance of protein, carbohydrate, and other constituents from a mixture of

Table 1. Influence of protein level on N:P factor (Tkachuk, R., unpublished data).

	Pearl millet	Sorghum	Chick-pea	Teff
Protein (1)	9.5	7.0	19.3	10.1
N:P	5.36	5.58	5.53	5.31
Protein	17.1	14.6	23.5	13.4
N:P	5.49	5.62	5.44	5.42

Note: N × 5.7 dry basis.

Table 2. Influence of N:P factor on reported "protein" level.

	"Protein" at different % of N (Kjeldahl)				
	2	3	4	5	6% N
N:P factor					
5.2 (A)	10.4	15.6	20.8	26.0	31.2
5.7 (B)	11.4	17.1	22.8	28.5	34.2
6.0 (C)	12.0	18.0	24.0	30.0	36.0
6.25 (D)	12.50	18.75	25.0	31.25	37.5
Diff. D-A	2.10	3.15	4.20	5.25	6.30

materials, most of which differ in amino acid composition. Because amino acids differ both in their individual composition and in their distribution within proteins, most proteins themselves differ in nitrogen content. As a result, the N:P factor differs from crop to crop, and in most instances the factor of 6.25 will lead to spuriously high values for the "protein" figures. Diets and feed formulations based on these figures are unlikely to provide protein nutrition to conform with their apparent composition. A recent study on three cereals and a legume fed at different protein levels revealed that the N:P factor differed within a crop as a direct result of differences in amino-acid composition (Tkachuk, R., unpublished data). This study is summarized in Table 1. Not only did the N:P factors differ at different protein levels, but they differed in different directions.

The influence of the N:P factor on the "protein" content is illustrated in Table 2. The effect is mathematically most marked in materials with high nitrogen contents. Although legumes are regarded as reliable sources of high protein for human nutrition, this observation is of particular concern to breeders and nutritionists in the fields of legume production and utilization.

The most significant nitrogen-containing substances in protein synthesis and metabolism in nonruminant animals (including humans) are the amino acids, and certain amides, such as aspara-

gine and glutamine. Consequently, the most logical method of arriving at a N:P factor is to determine the total amino acid and amide content of a commodity, and the respective distribution of amino acids and amide nitrogen. The total nitrogen content is then determined, and the ratio of total amino acids plus amides - total nitrogen in unit weight of sample gives the ratio N:amino acids plus amides, or the true N:P factor. The method of calculation is explained more fully in Appendix I. Maximum recovery of amino acids is essential, and this is affected mainly by hydrolysis conditions (6). Accuracy is also improved by meticulous observation of such conditions as the relative moisture contents of the samples used, respectively, for total nitrogen and amino acid determinations, and by correction for the elements of water added during hydrolysis. In most mature plant material the amount of soluble nitrogen (usually referred to as "nonprotein" nitrogen) is only about 2-3%, and consists largely of free amino acids, simple peptides, and intermediate compounds in protein metabolism. In other words, practically all of the nitrogen in plant material can be accounted for in terms of some type of amino acid, or derivative thereof. As the recovery of nitrogen as amino acids after hydrolysis is rarely in excess of 96%, it is likely that N:P factors based on amino acid analysis are slightly low. A reduction in recovery of amino acids of 3% means that a true N:P factor of 5.8

Table 3. Protein data for some pulses compared with wheat (Dronzek, B.L.: unpublished data).

Parameter	Lentil <i>L. esculatum</i>	Bean <i>V. faba</i>	Pigeon pea <i>C. cajanus</i>	Chick-pea <i>C. arietinum</i>	Vetch <i>V. narbonensis</i>	Vetch <i>V. gallilea</i>	Wheat <i>T. aestivum</i>
N:P factor ^a	5.48	5.26	5.76	5.55	5.46	5.50	5.68
Mean protein ^b	28.1	28.1	23.0	21.9	29.1	26.4	14.1
High protein	36.4	34.2	25.8	26.0	33.3	—	—
Low protein	23.3	22.5	20.6	17.4	25.6	—	—
No. of samples	1688	511	40	2676	9	2	2
Lysine ^c	9.95	8.15	9.25	9.26	9.43	10.83	3.29
Histidine	5.23	4.45	6.88	4.64	4.27	4.58	3.82
Threonine	2.86	2.66	2.83	2.95	2.87	2.81	2.14
Valine	3.76	3.60	3.62	3.65	3.81	3.87	3.23
Methionine	0.34	0.35	0.61	0.73	0.35	0.33	0.77
Isoleucine	2.85	2.79	2.73	3.05	2.88	2.98	2.34
Leucine	5.14	5.09	5.34	5.45	5.11	5.42	4.70
Tyrosine	1.44	1.21	1.13	1.24	1.33	1.24	1.41
Phenylalanine	2.76	2.27	5.16	3.21	2.45	2.47	2.75

^aCorrected to 98% recovery of amino acids.

^bN × 5.7, dry basis. Standard error of check sample analysis (chick-pea) = 0.263%.

^cGrams amino acids per 100 g protein.

will be reported as 5.62, for example.

Table 3 contains some details of the protein makeup of some common and less common legumes (6). Due partly to the absence of tryptophan and cystine figures, the recoveries were on the average rather low (89–90%), and for the purpose of this table, the figures were adjusted proportionally for each amino acid, to comply with a recovery of 98%. The data for wheat agrees well with literature values (2). Figures for total protein and some essential amino acids are included. All of the legumes provide an excellent source of lysine, compared with wheat. The legumes cited all provide better sources than wheat for all of the essential amino acids listed with the exception of methionine and tryptophan. The single sample of vetch *Vicia gallilea* was particularly rich in lysine. Lentil and bean (*Vicia faba*) were the richest sources of total protein.

The foregoing remarks underline the facts that (a) N:P factors vary widely between species of legume (as well as other commodities), and that, (b) none of the N:P factors are as high as 6.25. For all practical purposes, variation in the N:P factor is of no serious consequence, provided that the factor is quoted at the same time as the protein figures derived from it. The nutritionist and feed compositor can either compute food and feed mixes on the basis of total nitrogen, or

convert, for example, $N \times 5.7$ "protein" figures to $N \times 6.25$ values to comply with practiced procedure in specific operations.

A worldwide campaign to standardize the N:P factor would in all probability spark a controversy that would endure until the turn of the century. However, were such a campaign to be waged, it would be more realistic to establish that the factor of 5.7 should serve as the standard N:P factor, for the reporting of protein content in all commodities of plant origin likely to be involved in the formulation of foods and feeds for human and other animals.

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Appendix Table I. Calculation of N:P factor (includes typical values for series I for beans). Original protein content of sample = 23.7% ($N \times 5.7$ dry basis).

AA	G AA N/ 100 g total N, I	K_{AA} ($N \times 5.7$)	K_{AA} ($N \times 6.25$)	G AA residues/ 100 g sample II
Tryptophan	0.482	0.8575	0.9400	0.562
Lysine	8.343	1.2450	1.3651	6.702
Histidine	4.583	1.7455	1.9139	2.626
NH ₃	10.683	4.6877	5.1403	2.279
Arginine	21.584	2.0424	2.2395	10.568
Aspartic acid	8.123	0.6932	0.7601	11.718
Threonine	2.738	0.7892	0.8650	3.469
Serine	3.518	0.9373	1.0278	3.753
Glutamic acid	10.001	0.6180	0.6777	16.183
Proline	3.270	0.8212	0.9005	3.982
Glycine	5.319	1.3987	1.5337	3.803
Alanine	4.313	1.1222	1.2304	3.843
Valine	3.601	0.8052	0.8829	4.472
Cystine	0.617	0.7186	0.7877	0.859
Methionine	0.342	0.6083	0.6669	0.562
Isoleucine	2.767	0.7048	0.7728	3.926
Leucine	5.001	0.7084	0.7728	7.096
Tyrosine	1.477	0.4896	0.5368	3.017
Phenylalanine	2.330	0.5425	0.5948	4.295
Total series II				93.715
Total II \div (100 \div 23.7) = 93.715 \div 4.2194 = 22.2105 = III				
Total III \div % N in sample = 22.2105 \div 4.1579 = 5.34 = N:P factor				

Appendix

Calculation of N:P Factor

1. The amino acid (AA) analyzer should be programmed 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.

$$\text{Grams AA residues/100 g protein} = \text{II.}$$

The K_{AA} constants for all common AAs are listed in Appendix Table I for $N \times 5.7$ and $N \times 6.25$.

3. Total series II and divide by $(100 \div \% \text{ protein in original sample})$, to arrive at $\% \text{ AA residues in original sample} = \text{III.}$

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

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