Nitrogen-to-Protein Conversion Factors for Cereals and Oilseed Meals[1]

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ABSTRACT

Nitrogen-to-protein conversion factors were calculated for cereals and oilseed meals from quantitative amino acid data. The resulting values, varying from 5.4 to 5.8, indicate that the commonly used factors of 5.7 and 6.25 can overestimate the total protein content of cereals and oilseeds.

The protein content of common cereals and oilseeds is an important criterion of their food or feed value, since proteins are essential for the existence and growth of virtually all animal life. The quantity of protein in cereals and oilseeds is calculated from the amount of nitrogen in these materials, nitrogen content usually being determined by the Kjeldahl method. To calculate the amount of protein present, the nitrogen content is customarily multiplied by a factor of 5.7 or 6.25. The 6.25 factor is used for most feed materials; the practice apparently originated from early research on proteins of animal origin which were found to contain approximately 16% nitrogen (100 ÷ 16 = 6.25). The value of 5.7 is usually applied in many countries for wheat used for food purposes, and its use is apparently derived from the careful work by Osborne on the amount and nitrogen content of gliadin and glutenin in wheat (1).

The practice of using 5.7 and 6.25 as factors in calculating protein content is, however, based on an incorrect assumption and a number of erroneous conclusions. The incorrect assumption is that proteins contain either 16 or 17.5% of nitrogen (16 = 100 ÷ 6.25; 17.5 = 100 ÷ 5.7). That this assumption was incorrect was recognized in 1931 by D. B. Jones, who calculated more accurate nitrogen-to-protein factors by taking into account the fact that different plant proteins contain various amounts of nitrogen (2). Jones's calculations yielded values varying from 5.18 to 6.25 for many food substances (2).

While the calculations by Jones were an important advance in the knowledge of protein content of substances, his calculations are in error because of an erroneous procedure and his disregard of nonprotein N. The incorrect procedure is to calculate the nitrogen-to-protein factor from the nitrogen content of a protein or proteins of major occurrence, as they do not represent all of the proteins that are present, and in addition, there are no criteria of purity available for these proteins (e.g., wheat glutenin, barley hordein, oat prolamin). Such criteria of purity probably will never become available, as it is becoming apparent that the so-called major-occurring plant proteins are in reality very complex mixtures of different proteins, all of which can have different nitrogen contents, as has been shown for wheat (3,4,5). Jones realized that his disregard of nonprotein N was a limitation in the accuracy of his calculations, but was unable to correct for it because of lack of knowledge of nonprotein constituents in his day (2).

This paper is concerned with using quantitative amino acid data to calculate the nitrogen-to-protein factors for cereals and oilseeds, on the basis of an approach already used by the author for wheat flours (6). When that study was carried out (6), the author was not aware that Heathcote had used the same approach to calculate the nitrogen-to-protein factor for oats in 1950 (7).

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MATERIALS

All calculations carried out in the present work are on materials described in a previous article (8).

METHODS AND RESULTS

To illustrate the method of calculating the nitrogen-to-protein factor, a complete calculation is illustrated for Manitou wheat in Table I. It is seen that for 0.1754 g. of Manitou wheat N the sum of the weights of the recovered amino acid residues is 942.6 mg., and that the corresponding weight of N in these amino acid residues is 168.0 mg. The nitrogen-to-protein factor is then equivalent to 942.6 ÷ 168.0 or 5.61.

Similar calculations were carried out for other cereals and oilseeds for which complete and quantitative amino acid composition data were available, including accurate estimates of ammonia contents arising from amide hydrolysis (8). These calculations are summarized in Table II.

In the method used to calculate the nitrogen-to-protein factors in the present work, there is an insignificant error because the "anyhydro amino acid" definition for protein was applied to all the amino acid residues that are present. In reality, this definition should not be applied to the end groups of the proteins, as they are not entirely in the anhydro form. However, as the relative number of end groups is small in comparison to the total number of amino acid residues, the magnitude of the error is also small, being equal to:

$$\frac{[(400-2)(132-18)+(132-17)+(132-1)]-[400(132-18)]}{[(400-2)(132-18)+(132-17)+(132-1)]}\times 100$$

or 0.035%, assuming that one amino and carboxyl end group is present for every molecular-weight (MW) unit of 45,600, and that the average amino acid MW unit is 132, as it is for wheat (8).

DISCUSSION

It is seen from Tables II and III that the values for nitrogen-to-protein factors of cereals and oilseeds range from 5.26 to 5.76. The factors can be calculated from quantitative amino acid data with reasonable precision as is illustrated in Table II, where two different samples of wheat and oats gave similar results. These results indicate that the practice of using the factors 5.7 and 6.25 is untenable because they overestimate the total protein content of cereals and oilseeds. The factor of 6.25 in particular overestimates the protein content; for example, when used for wheat bran or soybean it may overestimate the protein content by 9.7 and 18.8%.

As shown in Table III, the conversion factor of 5.50 obtained in the present work for oats is in reasonable agreement with the factor of 5.40 obtained by Heathcote (7), considering that Heathcote carried out the work in 1950 when not all of the necessary precautions for hydrolyzing proteins in order to obtain quantitative amino acid recoveries were known, and considering also that he used a microbiological method for amino acid analysis which is usually considered less accurate than the automatic ion-exchange method.

TABLE I. CALCULATION OF THE NITROGEN-TO-PROTEIN FACTOR FOR MANITOU WHEAT

	Amino Acid Content μM/g. protein[a]	Weight, Nitrogen mg./g. protein[a]	Weight, Amino Acid Residue mg./g. protein[a]
Tryptophan	79	2.21	14.71
Lysine	177	4.96	22.69
Histidine	162	6.81	22.22
Ammonia	2,280		
Arginine	246	13.78	38.42
Aspartic acid[b]	299	4.19	34.41
Asparagine[b]	108	3.03	12.32
Threonine	253	3.55	25.58
Serine	495	6.94	43.10
Glutamic acid[b]	299	4.19	38.60
Glutamine	2,172	60.86	278.30
Proline	1,029	14.42	99.93
Glycine	559	7.83	31.89
Alanine	418	5.86	29.70
Cystine	110	3.08	22.47
Valine	416	5.83	41.24
Methionine	124	1.74	16.27
Isoleucine	291	4.08	32.93
Leucine	552	7.73	62.46
Tyrosine	168	2.35	27.41
Phenylalanine	326	4.57	47.98
		167.99	942.61

Amino acid N recovery: 95.8%
Factor: 942.61 ÷ 167.99 = 5.61

[a]Protein = N X 5.7.

[b]The amounts of ammonia, glutamic acid, and aspartic acid found were 2,280, 2,471, and 407 μM/g. protein. As the amounts of the carboxylic acids are in excess [(2,471 + 407) − 2,280 = 598], the free carboxylic acid residues were assigned equally to glutamic and aspartic acid (598 ÷ 2 = 299).

TABLE II. CALCULATION OF NITROGEN-TO-PROTEIN FACTORS FOR CEREALS AND OILSEED MEALS

	Weight recovered from sample containing 0.1754 g. N		Amino acid N recovery	Nitrogen-to-protein factor
	Amino acid residue mg.	Amino acid nitrogen mg.	%	
Manitou wheat	942.6	168.0	95.8	5.61
Selkirk wheat	947.3	168.9	96.3	5.61
Wheat flour	960.9	171.9	98.0	5.59
Wheat germ	793.9	145.8	83.1	5.45
Wheat bran	729.4	138.6	79.0	5.26
Rye, dark flour	885.7	157.2	89.6	5.64
Triticale	962.4	167.2	95.3	5.76
Barley, six-row	899.0	158.7	90.5	5.67
Barley, pot	984.9	173.3	98.8	5.68
Oats, hulled (A)[a]	893.9	162.5	92.7	5.50
Oats, hulled (B)[b]	902.1	163.9	93.4	5.50
Soybean meal	911.1	160.1	91.3	5.69
Buckwheat	866.7	156.8	89.4	5.53
Millet	980.4	172.6	98.4	5.68
Mustard meal	849.2	157.2	89.6	5.40
Rapeseed meal	892.9	161.5	92.1	5.53
Sunflower, hulled, meal	914.9	170.7	97.3	5.36
Flax meal	901.0	166.6	95.0	5.41

[a]Crop average for 1963.

[b]Commercial sample.

TABLE III. NITROGEN-TO-PROTEIN FACTORS FOR CEREALS AND OILSEED MEALS

	Present work	Literature values		
		Jones (2), USDA (10)	Heathcote (7)	Ewart (9)
Wheat	5.66[a]	5.83	.....	5.68
Wheat flour	5.59	5.70	.....	.....
Wheat germ	5.45	5.80	.....	.....
Wheat bran	5.26	6.31	.....	.....
Barley	5.67	5.83	.....	5.80
Barley, pot	5.67[b]	.....	.....	.....
Oats, hulled	5.50[b]	5.83	5.40	5.71
Rye flour	5.64	5.83[c]	.....	5.79
Soybean meal	5.69	5.71	.....	.....
Buckwheat	5.53	.....	.....	.....
Millet	5.60	.....	.....	.....
Mustard meal	5.40	.....	.....	.....
Sunflower, hulled, meal	5.36	5.30	.....	.....
Rapeseed meal	5.53	.....	.....	.....
Flax meal	5.41	.....	.....	.....

[a]Average for Selkirk and Manitou wheat, and Triticale from Table II.

[b]Average values from Table II.

[c]For rye.

The conversion factors of 5.59, 5.67, 5.50, and 5.64 for wheat flour, pot barley, hulled oats, and dark rye flour differ slightly from the values of 5.68, 5.79, 5.80, and 5.71 reported by Ewart (9) for wheat, barley, oat, and rye flours. Differences in values of conversion factors must be related to differences in amino acid compositions, since the two quantities are directly related. Higher relative amounts of lysine, histidine, arginine, glycine, alanine, and particularly ammonia will lower the value of the conversion factor, whereas higher relative amounts of tryptophan, aspartic acid, glutamic acid, methionine, isoleucine, leucine, tyrosine, and phenylalanine will raise the value. Examination of the amino acid data used by Ewart to calculate the conversion factors (9) reveals differences between his data and ours (8). For example, comparing Ewart's results for barley flour with ours for pot barley shows that differences of −15, +14, +25, +15, +11, +28, +19, +25, +14, −65, +20, +23, and +21% are present for tryptophan, lysine, histidine, ammonia, arginine, aspartic acid, threonine, glycine, alanine, cystine, valine, isoleucine, and tyrosine. The differences were calculated thus: [(value our work) − (Ewart's value)]/,[(value our work)] X 100. These differences in the amino acid composition result in the different values of 5.67 and 5.79 for the conversion factor for the barley products. The differences in the conversion factors reported by Ewart (9) and our results (8) for wheat, oats, and rye can be explained similarly.

It must be noted that protein content, as defined in the present work, includes all the amino acid content minus the elements of water:

$$n(NH_2\text{-}\underset{\displaystyle R}{\underset{|}{CH}}\text{-}COOH) = n(-NH\text{-}\underset{\displaystyle R}{\underset{|}{CH}}\text{-}CO-) + nH_2O$$

This definition includes free amino acids and peptides as forming part of the total protein content. It is appropriate that total protein content in cereals and oilseeds should be defined as the sum of the proteins and the small amounts of amino acids and peptides that are present, since cereal and oilseed protein is used mainly for

nutritive purposes, and it is known that all organisms must utilize proteins in the hydrolyzed form of amino acids and peptides.

It should be noted that the present work does not allow for the effect of nonprotein N on the nitrogen-to-protein factors; allowing for nonprotein N would reduce their value. However, it is not possible to estimate accurately the extent of reduction, since the amount of nonprotein N in cereals and oilseeds is not known at the present time. An approximate correction for nonprotein N can, however, be estimated for materials like wheat bran, wheat germ, buckwheat, mustard, and rye, which seem to contain larger amounts of nonprotein N (Table II). This may be done by multiplying the factors by the amino acid N recoveries in Table II. Thus the corrected factor for wheat bran would be equal to 5.26 X 0.79 or 4.16.

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