NITROGEN FACTORS

AAFCO’s Laboratory Methods & Services Committee
Moisture Best Practices Working Group

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Default 6.25 factor

100 / 16% N

Established in the 1880’s

based upon pure proteins readily available
serum albumin and globulin from blood
casein from milk
Few matrices contain exactly 16% N in their protein structure

D.B. Jones’s USDA Circular 183 in 1931

established a listing of nitrogen:protein factors for a variety of cereal grains, oilseeds, nuts, and other food commodities

Improved technologies and other researchers have shown some of the Jones factors to be in error.
Soy protein

5.71 -- Jones stated that major protein in soybeans is glycinin, a globulin composed of 17.5% N

glycinin is only one of the proteins in soy

amino acid data give factors with ranges of 6.24 – 6.37
removed tables of factors because AOCS does not have information on protein composition

The position was that the use of conversion factors should be a contractual agreement between buyer and seller, and not a scientific one.

6.25 for soy trade
Amino acid profiles are now more frequently used to determine protein content.

Tkachuk conducted 24, 48 & 72-hour hydrolysis

Amide N from asparagine and glutamine

Mosse et al used the sum of the anhydrous amino acid residues to calculate N factor for each amino acid

Anhydrous amino acid residue = each amino acid loses one molecule of water when they are joined to form dipeptide linkages

FAO recommended in 2002 to measure protein as the sum of individual anhydrous amino acids
Correction for Non-Protein Nitrogen

nucleic acids, amines, urea, biuret, ammonium compounds, nitrates, nitrites, vitamins, alkaloids, phospholipids, and nitrogenous glycosides

To the extent possible, the N content of these compounds needs to be subtracted from the total N content when using a factor
Use of Mass Balance to Evaluate Accuracy of Protein Values

sum of H2O, protein, fat, ash & CHO = 100
(where CHO is determined from NDF, sugars & starch)

Caldwell at Midwest Labs obtained protein of 100.1% using 6.25 factor on dried beef stock.

Protein was then calculated by subtraction and value of 90.75% obtained.

Amino acid analysis showed that actual protein content was 90.35%. Factor should have been 5.64.
RECOMMENDATIONS

Use Amino Acid Analysis to Determine Protein or Nitrogen-Protein Conversion Factor

Cysteine/cystine & methionine by performic acid oxidation tryptophan by base hydrolysis hydroxyproline in meat and collagen products

Anhydrous amino acid residues are summed to estimate protein or to calculate the conversion factor.
RECOMMENDATIONS

Use Appropriate Nitrogen:Protein Factor

FAO & AOCS – 6.25 for soy and vegetable protein products

Dairy industry – 6.38 for dairy products
Milk replacer if dairy based use 6.38; if soy based use 6.25

Wheat – 5.70

Other products – default factor of 6.25
RECOMMENDATIONS

Use Common Logic

Does protein value obtained seem reasonable

Was any non-protein nitrogen that can be determined (such as urea and ammonia) subtracted from total N

Calculate mass balance (if CHO are absent or if NDF, sugars & starch were determined) = 100

Have another individual look at the result and at product
NON-PROTEIN NITROGEN and EQUIVALENT CRUDE PROTEIN

N from urea, biuret, and ammonium salts added to ruminant feed is collectively known as *non-protein nitrogen* (*NPN*).

Manufacturers required to list *equivalent crude protein* (*ECP*) content on label.

\[ \text{ECP} = \text{NPN} \times 6.25 \]
NON-PROTEIN NITROGEN and EQUIVALENT CRUDE PROTEIN

NPN & ECP both determined by same method. Confusion in how results are reported.

Urease titration method measures urea & ammonium compounds as NH3 and results are expressed as N.

Results from method are reported as NPN.

NPN must by multiplied by 6.25 to report as ECP.

There is no feed method to measure biuret.
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Questions?