# Report on Nitrogen Factors and Animal Feeds

AAFCO's Laboratory Methods and Services Committee
Nitrogen Factors Best Practices Working Group
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The Nitrogen Factors Best Practices Working Group was directed by AAFCO's Laboratory Methods and Services Committee to review the use of nitrogen factors for determining protein in animal feeds and to make some recommendations.

## Background Discussion

Since the development of the proximate scheme of analysis at the German Weende Experiment Station in the 1860s and the development of the Kjeldahl method for nitrogen determination in the 1880s, protein has generally been determined by analyzing for nitrogen and multiplying the N content by a nitrogen:protein conversion factor (NPCF). The Weende proximate scheme assumed that all of the N came from protein. In the 1880s, pure proteins that were readily available for testing were serum albumin and globulin from blood and casein from milk. These were found to contain about 16% N. Dividing 100% by 16% give a NPCF of 6.25 and it was believed that this factor applied to all proteins.

As more research was done, it was found that few foods contain precisely 16% N and that the N content varies with plant species. D.B. Jones with the U.S. Department of Agriculture published USDA Circular 183 in 1931 (and slightly revised in 1941) that established a listing of NPCF for a variety of cereal grains, oilseeds, nuts, and other food commodities (1). These factors for specific commodities have been accepted and used over the years while the factor of 6.25 has been accepted and used for animal feeds that contain a mixture of ingredients.

With improved analytical technologies, it has been found that some of the factors published by Jones and others were not accurate. The values used are partially conventional and frequently erroneous because they are based on inaccurate data and on forgotten assumptions (2). Researchers have also shown there are variations in N content amongst cultivars in a species. (2, 3).

One example is that of soybeans. Jones justified his 5.71 NPCF for soybeans by stating the major protein in soybeans is glycinin, a globulin composed of 17.5% N. Research has shown the glycinin is only one of the proteins in soy (4). Analytical data of amino acids for over 50

samples of various soy products conducted by the U.S. Department of Agriculture, independent laboratories, and an independent researcher show a NPCF in a range of 6.24 – 6.37 (5).

Sriperm et al determined that the NPCF of 6.25 overestimated the true protein of 5 different feedstuffs (6). She concluded that the appropriate factors should be: 5.68 for corn, 5.64 for soybean meal, 5.74 for corn DDGS, 5.45 for poultry by-product meal, and 5.37 for meat and bone meal.

Tkachuk stated that the NPCF of 5.7 should be applied to wheat used in animal feed instead of the traditional 6.25 factor used for feeds. (7)

The American Oil Chemists Society (AOCS) convened an expert panel to review AOCS methods for N and protein determination. The outcome was that the panel recommended that AOCS strip out all references to the Jones factors and to remove the tables of NPCF wherever they were listed in different methods. The decision was made although, AOCS supports the method of analysis for N, it does not have information on protein composition and cannot recommend conversion factors beyond stating that for soy trade the factor 6.25 should be used. The position was that the choice of conversion factors should be part of a contractual agreement between buyer and seller, and not a scientific one (8).

The use of incorrect NPCF can result in economic and environmental problems. An erroneously high NPCF will give an apparently higher protein value causing the consumer to pay more for protein that he/she is not receiving. Animals that consume more nitrogen than they utilize will excrete the excess N which can cause environmental pollution.

**Use of Amino Acid Profile to Determine Protein**

Amino acid profiles have been more frequently used to determine protein content. The work reported above was all based on amino acid data. Protein consists of at least 20 different amino acids bound together by peptide bonds between the carboxyl and amino groups of adjacent amino acids. Therefore true protein is the summation of the total amino acid residues from an amino acid analysis. These 20 amino acids are: alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Hydroxyproline from collagen needs to be included when considering meat products.

During acid hydrolysis, the amidated amino acids (asparagine and glutamine) which contain two nitrogen molecules are converted to their acidic counterparts which contain one nitrogen molecule. Some researchers have attempted to estimate the asparagine and glutamine

separately by trying to measure the amide ammonia coming from the second N molecule. Other researchers have included asparagine and glutamine as part of aspartic acid and glutamic acid.

Tkachuk conducted 24, 48 & 72-hour hydrolysis in order to ensure that all of the amino acids were liberated. He chose to use the highest values obtained in calculating his NPCF (7). If one wants to obtain truly accurate amino acid data, then these multiple hydrolysis times are needed. Most research and testing labs have established a single hydrolysis time that gives them an optimum amino acid recovery.

Mosse et al used the sum of the anhydrous amino acid residues to do his calculations (9). Each amino acid loses one molecule of water when they are joined to form dipeptide linkages and ultimately protein. Anhydrous amino acid residues are calculated by subtracting one molecule of water from the molecular weight of each amino acid. The N content of each amino acid residue is calculated from its molecular weight. A NPCF was calculated from the sum of the amino acid residues and the sum of the N content.

In December of 2002, the Food and Agriculture Organization convened the "Technical Workshop on Food Energy: Methods of Analysis and Conversion Factors". One of the significant outcomes of this workshop was this recommendation: *to measure protein as the sum of individual anhydrous amino acids, rather than the measurement of nitrogen by the Kjeldahl and other indirect methods* (10).

Animal nutritionists are moving towards the use of amino acid values or N values instead of protein values to do their ration balancing especially for monogastric animals.

**Correction for Non-Protein Nitrogen**

The original Kjeldahl method determines total N that comes mainly from protein and added ammonium compounds. The Dumas combustion method that is commonly being used today also determines total N in the test material. Feed matrices contain other nitrogenous compounds that are not protein. These non-protein nitrogenous compounds include nucleic acids, amines, urea, biuret, ammonia, nitrates, nitrites, vitamins, alkaloids, phospholipids, and nitrogenous glycosides. Some of these compounds can be present in a significantly large amount such as urea or biuret added into a ruminant feed. To the extent possible, the N content of these compounds needs to be subtracted from the total N content when using a NPCF to calculate protein.

Certain non-protein nitrogen containing materials have been used to adulterate foods (melamine in infant formula and pet foods in 2007). Estimating the protein content based on the determination of total N and using the NPCF commonly used for the pure ingredient allowed this adulteration to occur.

### Use of Mass Balance to Evaluate Accuracy of Protein Values

Researchers have also examined how NPCF factors affect the calculated protein value when looking at mass balancing. The sum of moisture, protein, fat, ash, and carbohydrates is to be a 100%. Jane Caldwell at Midwest Labs in Omaha NE analyzed 5 samples of dried beef stock and came up with a protein value of a 100.1% using the 6.25 NPCF (unpublished data). Caldwell knew that this product could not be pure protein. Protein was then calculated by subtraction from the proximate values and a value of 90.75% was obtained. Amino acid analysis of the beef stock showed that the actual protein content was 90.35% which agreed with the calculated 90.75% value. It was determined that the NPCF for this dried beef stock should be 5.64.

Similar work reported by FAO (10) on isolated soy protein showed that the traditional 5.71 NPCF for soy gave a protein value that was 8% lower than what was determined by amino acid analysis and using a NPCF of 6.25.

Mass balancing can be used on materials where protein content, moisture content, ash content, fat content and carbohydrate content have all been determined. Carbohydrates are determined by analyzing for neutral detergent fiber, sugars and starch, unless they are known to be zero.

## RECOMMENDATIONS

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

A complete amino acid profile for the *18 common amino acids* is conducted using an approved method such as AOAC 994.12. *Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, and valine* are determined by conducting a 24-hour acid hydrolysis in 6N HCl. *Cysteine/cystine* and *methionine* are determined by conducting a performic acid pre-oxidation following by acid hydrolysis. *Tryptophan* is determined by conducting a basis hydrolysis as in AOAC 988.15. *Hydroxyproline* also needs to be determined with meat products and other products with significant collagen levels (either by amino acid analysis or AOAC 990.26 or other approved method). The anhydrous amino acids residues are summed together to

calculate a better estimate of the protein content of the feedstuff. The sum of the N content of each anhydrous amino acid residue is used to calculate a nitrogen:protein conversion factor.

**Use Appropriate Nitrogen:Protein Conversion Factor**

Not every laboratory has the capability of conducting amino acid analyses and/or the time to do so on every sample. In these situations, the appropriate NPCF needs to be used when calculating protein based upon a N analysis. The appropriate NPCF is best determined by an amino acid assay. With a manufactured product or when the feed consists of a single ingredient (such as corn), an amino acid assay can be conducted on representative samples in order to calculate the NPCF. Protein is determined on subsequent samples via N analysis and the NPCF (as long as the ingredient composition of the manufactured product does not significantly change).

Some regulatory or commodity associations have established NPCF for specific products. Some of these are:

- FAO (United Nations Food and Agriculture Organization) and the AOCS (American Oil Chemists Society) recommends that **6.25 be used for soy and vegetable protein products** (8, 11)
- Dairy Industry uses **6.38 for all dairy products** (12) Note: Use 6.38 if a milk replacer is dairy based; use 6.25 if it is soy based.
- For **wheat use 5.7** (7)
- **Default factor of 6.25** can be used on all other products.

**Use Common Logic**

Does the protein value obtained seem reasonable when using a given NPCF?

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

If the material has been characterized for protein, moisture, ash, fat and carbohydrates, mass balance can be used. It should approximate 100%. If it is not close to 100%, some of the estimations are incorrect.

Have another individual look at the result and at the product.

# NON-PROTEIN NITROGEN and EQUIVALENT CRUDE PROTEIN

At times there is confusion between the terms non-protein nitrogen (NPN) and equivalent crude protein (ECP).

Urea, biuret, and sometimes ammonium salts can be added to ruminant feeds as a nitrogen source. Urea entering the rumen is rapidly hydrolyzed by bacterial urease to ammonia. The rumen microorganisms utilize the ammoniacal nitrogen to synthesize protein. The nitrogen from urea, biuret, and ammonium salts is collectively known as *non-protein nitrogen (NPN)*.

Manufacturers are required to inform the customer that NPN has been added to the feed. Manufacturers do this by listing an *equivalent crude protein (ECP)* content on the label. Equivalent crude protein is determined by multiplying the NPN content by 6.25.

NPN and ECP are both determined by the same analytical method, generally a version of AOAC 941.04 whereby a feed test portion is incubated with urease to convert urea into ammonia. The ammonia is then distilled over into an acid solution and titrated. (Note: This method does not detect biuret. Unfortunately, there is not a method to measure biuret in feeds. There is one for fertilizers.)

The confusion is in how the results are reported. Ammonia is measured during the titration process and the results are expressed as N; so the results from the titration are reported as NPN and not as ECP. The NPN value must be multiplied by 6.25 to report the results as ECP.

## References


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