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Evaluation of nitrogen conversion factors for dairy and soy

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EVALUATION OF NITROGEN CONVERSION FACTORS FOR DAIRY AND SOY

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FOREWORD

High quality protein is an essential component of balanced nutrition for all age groups. Scientifically robust methods must be used to determine both the amount and quality of the protein. This is essential for optimization of the protein in diets and for optimization of resources, such as land, water and energy, used to produce this protein.

IDF supports efforts that aim to maintain accurate determination of protein levels using methods and, where appropriate, nitrogen conversion factors (NCFs) that are scientifically based for all proteins in foods. Over the years, NCFs have been discussed by various committees of the Codex Alimentarius Commission. IDF has contributed to these discussions by providing science-based advice and insights, including a comprehensive review of scientific literature pertaining to nitrogen conversion factors for a range of foodstuffs (IDF Bulletin 405, 2006).

Current and future challenges relating to nourishing a growing world population mean that protein, as one of the principal nutrients of human food, will remain in the spotlight. Hence, the measurement of protein, including the use of appropriate NCFs, is expected to be a subject of on-going interest. The current publication was prepared by the IDF *Task Force on Nitrogen Conversion Factors* and aims to make an important contribution to such discussions by the Codex Alimentarius Commission through an updated review of the scientific literature relating to dairy and soy, and by presenting some new data relating to these product groups.

IDF would like to thank the leader of the Task Force “Nitrogen Conversion Factor”, Dr. Jaap Evers (IDF), and all the members of the Task Force for preparing this extensive work: Mrs. Iraz Alper (FR), Dr. Dave Barbano (US), Ms. Melissa Cameron (AU), Dr. Bita Farhang (CA), Dr. Marina Foa Gips (IL), Prof. Dr. Hermann Frister (DE), Mr. Christophe Fuerer (CH), Mr. Roger Hall (NZ), Mr. Claus Heggum (DK), Dr. Jeremy P. Hill (NZ), Dr. Marieke Lugt (NL), Mr. Juan Romero (US) and Dr. Jan M. Steijns (NL).

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Brussels, January 2016

1 SUMMARY

The protein content of foods is commonly calculated by multiplying the analytically measured nitrogen content of a sample of food by a so-called nitrogen conversion factor (NCF). The use of scientifically appropriate NCFs for different foodstuffs is important for nutritional, sustainability and regulatory purposes.

The 37th session of the Codex Committee on Methods of Analysis and Sampling (CCMAS) (Budapest, 22–26 February 2016) has been asked to assess the accuracy and appropriateness of an NCF value of 5.71 for:

- determination of protein content in soybean products in general;
- soy protein used in formula for infants and young children taking into account the amino acid profile of the isolate.

About a decade ago, Codex considered the NCFs for both soy products and milk products. IDF contributed to the Codex deliberations at that time by sharing its findings from a review of the literature [1], which concluded that there was no scientific justification to change the NCF for soy from 5.71 to 6.25, or that for milk protein from 6.38 to 6.25. In light of the current discussions within Codex, IDF has provided in the current Bulletin an updated review of the scientific literature for both soy proteins and milk proteins, and also presented some new data.

Key conclusions

- The overwhelming consensus of scientific studies is that specific NCFs for specific foods should be used.
- For both dairy protein and soy protein, scientific publications based on experimental and/or theoretical analysis of NCFs consistently demonstrate that use of an NCF of 6.25 is incorrect and scientifically flawed.
- For soy products in general, the scientific literature reports NCFs in the range 5.6–5.8. The only value quoted higher than this range (6.30, for soy flour) was obtained through erroneous exclusion of nitrogen content from the amides contained in asparagine and glutamine.

- The data for soy protein isolates reported in the scientific literature indicate that the NCFs for these products (range 5.63–5.85; mean 5.73) are not substantially different from those for other soy products.

- The limited data for soy hydrolysates reported in the scientific literature indicate that the NCFs for these products (range 5.56 – 5.59; mean 5.58) appear to be similar to those for other soy products.

- Allowing for wide variation in the ratio of 11S to 7S proteins from different soy cultivars, the calculated NCFs for soy-based infant formulas range from 5.69 to 5.79 (mean 5.74). This mean is very close to the value of 5.71 stated in the Codex Standard for Infant Formula [2] as applicable to soy-based infant formula.

- For milk-based infant formulas, allowing for (1) different ratios of whey protein to casein in the final product and (2) wide variation in whey protein composition as a result of different manufacturing processes, the calculated NCFs range from 6.30 to 6.50 (mean 6.39). This mean is very close to the value of 6.38 stated in the Codex Standard for Infant Formula as applicable for milk-based infant formula [2].

2 INTRODUCTION

Protein is a principal nutrient in the human diet and its content in foods is commonly measured by determining the amount of nitrogen and multiplying that by a specific factor, the nitrogen conversion factor[1] (NCF). The NCF can be determined by calculation from known protein composition and amino acid sequences, or by measuring the nitrogen content of a highly purified protein.

Hence, to formulate foods and to verify compliance with labelling requirements and other specifications, manufacturers and official control laboratories need to use scientifically justified NCFs that are ratified by international food standardization bodies such as the Codex Alimentarius Commission (CAC).

At its meeting in July 2015, the CAC decided to ask the Codex Committee on Methods of Analysis and Sampling (CCMAS) to "assess the appropriateness of the use of the conversion factor of 5.71 to determine protein content in soybean products in general" [3].

In view of this decision, the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) agreed to request CCMAS to "provide advice on the accuracy and appropriateness of 5.71 as the nitrogen factor for soy protein used in formula for infants and young children and to take into account the amino acid profile of the isolate" [4].

About a decade ago, Codex considered the appropriateness of NCFs for soy products and milk products. IDF contributed to the discussion at that time by reviewing the scientific literature [1]. In light of the current discussions within Codex, IDF has again reviewed the scientific literature as well as presented some new data; this work is described in the subsequent sections of the current Bulletin. Sections 3 and 4 provide information relevant to the questions raised by the CAC and CCNFSDU, respectively. In addition, for the convenience of interested parties, IDF has summarized the references to NCFs as found in Codex standards (Appendix 1). Furthermore, Appendix 2 provides a brief summary of current developments relating to protein quality, which stress the importance of the actual levels of indispensable amino acids in individual protein sources.

1 The nitrogen conversion factor may also be referred to by other terms such as "protein conversion factor" and "nitrogen-to-protein conversion factor".

3 REVIEW OF THE LITERATURE

3.1. Summary of IDF Bulletin 405

IDF Bulletin 405 [1] reviewed the literature relating to NCFs for a range of foodstuffs. It concluded:

There exists no scientific justification to support the change of the original protein source nitrogen conversion factor for soy from 5.71 to 6.25, or to change the established nitrogen conversion factor for milk protein from 6.38 to 6.25.

This conclusion was based on the assessment of scientific publications detailing experimentally determined NCFs from analysed samples and/or theoretical NCFs calculated from amino acid data (summarized in Table 1).

Product	NCF	Reference
Milk	6.38	[5]
Milk	6.38	[6]
Milk	6.34[a, b]	[7]
Milk	6.35[c]	[8]
Skim milk powder	6.91[d] 6.13[e]	[9]
Milk and milk products	5.94[f]	[10]
Soy	5.71	[6]
Soy flour	5.71	[11]
Soy products	5.71	[11]
Soy	5.75–5.8	[12]
Soy isolate	5.6–5.8	[12]
Soy - commercial defatted flakes	5.66[g]	[13]
Soy - experimental acid precipitate isolate	5.77[g]	[13]
Soy - Experimental dialysis isolate	5.80[g]	[13]
Soy - commercial isolate	5.70[g]	[13]
Soy meal	6.30[d] 5.65[e]	[9]

Product	NCF	Reference
Soybean	k_A = 5.67[h] k_P = 5.38[i] k = 5.52[j] (the author claimed this to be the best estimate of the true NCF for soybean)	[14]

Table 1. NCFs cited in IDF Bulletin 405 [1] from studies on the NCF for dairy protein and soy protein based on experimental analyses of samples and/or theoretical calculations based on amino acid data. In some cases, additional data from the original papers has been added for a more comprehensive overview

[a] A wide range of factors were reported for milk, milk products and individual milk proteins. For simplicity's sake we reproduce here only the factor for milk. The reader can access all the other factors in the original publication [1].
[b] This value was corrected by van Boekel and Ribadeau-Dumas [8].
[c] The authors state that because of some uncertainties regarding some serum proteins and the carbohydrate portion of κ-casein the true value may be in the range of 6.34–6.36.
[d] Excluding nitrogen from asparagine and glutamine amide groups.
[e] Including nitrogen from asparagine and glutamine amide groups.
[f] The authors grouped different foodstuffs and calculated an **average** NCF for each group. Regretfully, in the analysed group where the dairy source data were averaged, the authors also included eggs (NCF 6.0), thus lowering the average NCF for dairy.
[g] Determined by the so-called "Factor Method" (dividing the sum of amino acid residue weights by the sum of their amino acid nitrogen content). A second method, the "Kjeldahl Method", involved dividing the sum of amino acid weights by the micro-Kjeldahl nitrogen content of the foodstuff. The results obtained by the Kjeldahl Method were very similar to those obtained by the Factor Method, namely 5.66, 5.76, 5.79 and 5.70, respectively. This study reported corrected values for those reported by the same author in 1981 [15], where subtraction of a water molecule from the amino acid molecular weight basis had been omitted, resulting in erroneously high estimates of NCFs, as well as poor agreement between the results of the Factor Method and those of the Kjeldahl Method.
[h] Determination was similar to the Factor Method, using the sum of anhydrous amino acid residues. Note that IDF Bulletin 405 [1] reported only a figure of 5.76, which appears to be a transcription error of the value of k_A.
[i] Determined by a principle similar to that used in the Kjeldahl method [13].
[j] The factor k can be estimated as follows: $k = (k_A + k_P)/2 \pm (k_A - k_P)/4$.

3.2. Recent review of the literature

In the current Bulletin, IDF presents a new literature review focusing on papers dealing with the determination of NCFs. This literature search yielded some papers from the period prior to 2006 that had not been mentioned in IDF Bulletin 405. In addition, several papers dealing with NCFs for dairy and soy that had been published in the period 2006–2016 were found and are included in Table 2. Because the current review focuses particularly on studies dealing with dairy or soy, NCFs reported for other foodstuffs (for example, [16-20]) are not included in Table 2. Infant formula is included because this product uses milk protein and/or soy protein as ingredients, and because the issue of the NCF for determining the protein content of soy-based follow-up formula is currently a point for discussion within Codex.

Foodstuff	NCF[a]	**Comments**	**Reference**
Cow's milk	6.32[b]	-	[12]
Cow's milk casein	6.37[b]	-	[12]
Cow's milk	6.02[c]	-	[21]
Casein	6.15[c]	-	[21]
Cheddar cheese	6.13[c]	One sample	[21]
Cheddar cheese	6.39	48 samples	[22]
α_{s2}-Casein	6.30[d]	6.06[e]	[23]
β-Casein	6.37[d]	6.28[e]	[23]
κ-Casein	6.35[d]	6.11[e]	[23]
β-Lactoglobulin	6.29–6.38[d]	6.34[e]	[23]
α-Lactalbumin	6.26[d]	6.26[e]	[23]
Serum albumin	6.08[d]	6.08[e]	[23]
Casein	6.36[d]	6.22[e]	[23]
Milk protein	6.32[d]	6.19[e]	[23]
Milk	5.92[d]	5.80[e] Values calculated based on the seven main constituent proteins only and excluding NPN	[23]
Infant formula (milk based)	6.37	Calculated for a 20:80 whey protein to casein ratio	[24]
Infant formula (milk based)	6.38	Calculated for a 30:70 whey protein to casein ratio	[24]
Infant formula (milk based)	6.39	Calculated for a 50:50 whey protein to casein ratio	[24]
Infant formula (milk based)	6.39	Calculated for a 60:40 whey protein to casein ratio	[24]
Soybean meal	5.69	Determined for crude protein	[25]
Soybean	5.63	Determined for crude protein. Accounting for NPN reduced the NCF to a value of 5.22	[26]
Soybean/soybean meal	5.50	Average value calculated from data by Sarwar et al [27] (5.64), Mossé [14] (5.52), Tkachuk [25] (5.44) and Morr [13] (5.40)	[23]

Foodstuff	NCF[a]	Comments	Reference
Soybean meal[f]	k_A 5.64 k_P 5.13 k 5.39	NCF definitions based on Mossé [14]. Note that total N was determined according to the Dumas method instead of the Kjeldahl method. Mossé [14] concluded that *k* gives the best estimate of the true NCF. Mariotti et al. [23] are of the opinion that k_A should be preferred for *purified* protein (where the amount of non-protein nitrogen (NPN) is low) such as purified protein products extracted from milk and soybean), but that k_P is preferred when assessing the protein content from the nitrogen content. However, it is noted that k_P depends on the analytical recovery during amino acid recovery and thus tends to underestimate the protein content. Sriperm et al. [28] conclude that k_A is the best estimate of the NCF for determining true protein.	[28]
Soy β– conglycinin (α’)	5.58[g]	7S protein subunit; calculated from protein structure based on data from Utsumi et al. [29]	[24]
Soy β– conglycinin (α)	5.65[g]	7S protein subunit; calculated from protein structure based on data from Utsumi et al. [29]	[24]
Soy β– conglycinin (β)	5.66[g]	7S protein subunit; calculated from protein structure based on data from Utsumi et al. [29]	[24]
Glycinins (mean value of the five subunits)	5.56[g]	11S protein; calculated from protein structure based on data from Utsumi et al. [29]	[24]
Soy cultivar 1	5.79[h]	Calculated for a cultivar having a ratio of glycinin (11S) to β-conglycinin (7S) of 0.5	[24]
Soy cultivar 2	5.73[h]	Calculated for a cultivar having a ratio of 11S to 7S of 1.0	[24]
Soy cultivar 3	5.69[h]	Calculated for a cultivar having a ratio of 11S to 7S of 1.5	[24]

Table 2. Additional NCFs for dairy protein and soy protein as reported in the literature, based on experimental analysis and/or theoretical computation

[a] Figures rounded to two decimal places.
[b] de Rham [12] reports different figures based on two different assumptions. Here the higher of the two values is quoted, as was done for soy in Table 1 and IDF Bulletin 405 [1].
[c] Calculated by excluding prosthetic groups.
[d] Calculated based on data from Farrell et al. [30] that included prosthetic groups. Calculated NCFs are essentially the same as those reported by van Boekel and Ribadeau-Dumas [8].
[e] Calculated from data from Farrell et al. [30] that excluded prosthetic groups. See section 3.3.1 for further discussion on this point.
[f] Dehulled solvent extracted soybean meal; ingredient for animal feedstuffs.
[g] Calculated by excluding prosthetic groups.
[h] Calculated by including prosthetic groups.

3.3. Discussion of results reported in sections 3.1 and 3.2

3.3.1. What does the term "nitrogen conversion factor" denote?

It is important to realize that the term "nitrogen conversion factor" does not necessarily denote the same thing in different studies, because the methodologies used by different authors for determining NCFs are not necessarily the same. The issue as to what the term "nitrogen conversion factor" means is intimately linked to the question of what is meant by "protein". This has been reviewed by Sosulski and Imafidon [21], Mariotti et al. [23] and Maubois and Lorient [24], and is likely to be the subject of further scientific debate. It is beyond the scope of the current review to repeat their considerations in detail; however, the following points are worth noting:

- For milk protein, Mariotti et al. [23] calculated two different NCFs:
  (1) *K*, for proteins *including* prosthetic groups;
  (2) *K'*, for proteins *excluding* prosthetic groups.
  Their rationale for computing *K'* was that *K* overestimates the potential of a source to provide amino acids when the peptide chains include prosthetic groups (glycosylated or phosphorylated residues).

  The use of *K'*, rather than *K*, reduces the apparent milk protein NCF from 6.32 to 6.19. This is a reduction of about 2% [23], or about 1% when using data by van Boekel and Ribadeau-Dumas [8].

  For soy products, applying the method proposed by Mariotti et al. [23] reduces the NCF to a value of 5.50 (average of data from four different studies [13, 14, 25, 27]; see Table 2), a reduction of about 4% compared with the traditionally used NCF of 5.71.

  However, Maubois and Lorient [24] point out that the prosthetic groups are constituent parts of the protein, because (1) they are covalently bound to the amino acid chain and (2) they possess nutritional, physiological and technological functions. These authors support their argument with the following examples:
  - κ-Glycomacropeptide, which is released through hydrolysis of casein micelles by rennet and pepsin in the stomach, regulates the differential bio-availability kinetics of caseins and whey proteins. This release also induces secretion of the cholecystokinin hormone implied in the regulation of gallbladder and pancreatic functions.
  - The cleavage of κ-glycomacropeptide from casein micelles is similarly involved in the phenomenon of milk coagulation by rennet, an essential step in cheesemaking.

- Whether or not to account for NPN in the calculation of the NCF for foodstuffs is a subject of debate. Sosulski and Imafidon [21] advocate accounting for NPN when establishing NCFs, because the NPN fraction may contain substantial proportions of free amino acids and peptides.

3.3.2. What scientific evidence has been reported to support NCF=6.25 for soy?

To the best of IDF's knowledge, no study has been published in the scientific literature that calculated an NCF of 6.25 for commercial soy products from theoretical or experimental data.

Mossé [14] pointed out that a NCF factor of 6.25 is never valid in plant material. Indeed, the current review of a wide range of studies across various foodstuffs, including many studies covering soy products, showed that the opinion of the various authors was very consistent in that they *rejected the use of a generic NCF of 6.25* [9, 10, 13, 14, 19, 20, 23-26, 28]. Instead, all these authors advocated the use of specific NCFs based on scientific grounds[2], which agrees with the recommendation of FAO [31].

3.3.3. Are the NCFs for soy isolates and hydrolysates substantially different from those for other soybean products?

Commercial soybean products are classified into three major groups: (1) flour and grifts; (2) concentrates and (3) isolates, having approximate protein levels of 40-54%, >70% and >90%, respectively, with the latter group supplying almost all the protein in liquid infant formulas [32]. Hence, it is worth assessing whether the NCFs for soy isolates and hydrolysates differ from those for other soybean products. Experimentally determined data obtained from studies in which different products were assessed are summarized in Table 3.

Study	Product	NCF
Sosulski and Sarwar [33]	Soybean meal	5.71
	Soybean isolate	5.74
de Rham [12][a]	Defatted soy flour	5.66
	Soy isolate 1	5.85
	Soy isolate 2	5.64
	Soy isolate 3	5.63
	Soy hydrolysate 1	5.59
	Soy hydrolysate 2	5.56
Morr [13]	Defatted flakes	5.66
	Experimental soy isolate (acid precipitated)	5.76
	Experimental soy isolate (dialysis)	5.79
	Commercial soy isolate	5.70

Table 3. Comparison of NCFs reported in the scientific literature for soy isolates/hydrolysates and other soybean products

[a] Experimental data from amino acid analysis, assuming 50% amidation

2 This excludes foodstuffs containing blends of different proteins or of which the protein composition is unknown. In these cases a factor of 6.25 has been proposed for practical reasons [21].

From the data in Table 3 it can be concluded that there is some variation in the estimates of the NCFs for soy isolates (range 5.63–5.85) and that the mean value of 5.73 is very close to the average value (5.68) for all soy products reported in these respective studies (Table 3). Furthermore, the mean value of 5.73 for soy protein isolates remains very close to that stated by Codex as appropriate for soy-based infant formula (5.71) [2].

The data support the conclusion by Mossé [14], who studied the effect of different methodologies for determining NCFs and stated, "…the present work shows that k_A is close to 5.7 for soybean proteins and this value is the real conversion factor for purified soy protein isolates". Hence, from the scientific literature there is no evidence that the NCF for soy protein isolates is substantially different from that determined for other soy products. Therefore, it appears that the use of 6.25 for soy isolates results in an overestimation of the protein content by about 8–9%.

The limited data for soy hydrolysates (Table 3) suggest that the NCF is similarly close to that of the defatted products, albeit somewhat lower. Again, the literature provides no scientific evidence to suggest that 6.25 is a justifiable factor for these products.

4
WHAT ARE APPROPRIATE NCFS FOR FORMULAS FOR INFANTS AND YOUNG CHILDREN?

4.1. Soy-based formulas

In the Codex working paper CX/NFSDU 15/37/5-Add.1 *Review of the standard for follow-up formula (Codex Stan 156-1987)* [34], the Federation of European Specialty Food Ingredients Industries (ELC) and the European Vegetable Protein Federation (EUVEPRO) state:

In 1931 (revised in 1941), USDA scientist D.B. Jones published a report ("Circular 183")[1] which proposed establishing unique nitrogen to protein conversion factors for several foods. Jones reported 5.71 as a more "precise" factor for soy protein. In this Circular[1], Jones hypothesized that not all nitrogen in foodstuffs was protein nitrogen and not all proteins contained 16% nitrogen; therefore, a universal conversion factor of 6.25 was not always appropriate. In support of his theory, Jones reported nitrogen contents for several plant and animal proteins from a variety of sources. Jones justified the 5.71 factor for soybeans by stating, incorrectly, that the major protein in soybeans is glycinin, a globulin composed of 17.5% nitrogen. From these data, he designated a conversion factor for soy protein of 5.71 (100 divided by 17.5 results in a factor of 5.71). Glycinin (11S), however, represents only about 31–52% of the total protein in soybeans[2-4]. There are many other proteins in soybeans, including beta-conglycinin (7S), which represents about 35% of the total protein[2-4]. ***If one considered only the 7S protein, the nitrogen to protein conversion factor for soy would be as high as 6.45[3,4]****. The ratios of 11S to 7S in soybeans will vary significantly, depending on the soybean variety and differences in seasonal growing conditions[2-4].*

[1] *Jones, DB (1931, slightly revised 1941) Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Protein. US Department of Agriculture Circular 183.*

[2] Murphy, PA and Resurreccion, AP (1984) Varietal and Environmental Differences in Soybean Glycinin and ß-Conglycinin Content. Journal of Agricultural Food Chemistry 32: 911-15.

[3] Roberts, RC and Briggs, DR (1965) Isolation and Characterization of the 7S Component of Soybean Globulins. Cereal Chem 42:71.

[4] Koshiyama, I (1968) Chromatographic and sedimentation behavior of a purified 7S protein in soybean globulin. Cereal Chem 45:405.

Roberts and Briggs [35] did not report a NCF for the 7S fraction they isolated. However, they did report that the nitrogen content of the protein isolated by them was 15.5%. We assume that ELC and EUVEPRO used this figure to calculate the NCF value they quote (100/15.5=6.45). This value could be inaccurate because of underestimation of the nitrogen content and/or overestimation of the protein content.

Koshiyama [36] reported neither an NCF value nor nitrogen data that would allow calculation of the NCF. Hence, this publication provides no independent support for an NCF of 6.45 for the 7S fraction.

Furthermore, ELC and EUVEPRO omitted to contrast the figure derived from the study by Roberts and Briggs [35] with those of later and more detailed studies on soy protein structure, such as those reviewed by Maubois and Lorient [24]. The latter authors calculated that NCFs for the three 7S soy protein subunits (α′, α and β, respectively) lie within a narrow range of 5.58–5.66 (mean 5.61) when excluding the prosthetic groups (Table 2). But, as mentioned in section 3.3.1, prosthetic groups should be included, and if this is done then the mean NCF for the 7S protein becomes 5.91. Hence, by taking into account the covalently bound prosthetic groups, Maubois and Lorient [24] calculated that NCFs for different soy cultivars with different ratios of 11S to 7S (0.5, 1.0 and 1.5) lie in the range 5.69–5.79 (Table 2). The mean value of 5.74 is very close to the 5.71 value stated in Codex Standard 72 [2], and clearly shows that factors of 6.45 and 6.25 would respectively overestimate the protein content in infant formula and follow-up formula by about 10–12% and 7–9%.

4.2. Milk based formulas

4.2.1. Effect of whey protein-to-casein ratio on the NCF for milk-based infant formula

The protein composition of milk-based infant formulas can differ in terms of the whey protein-to-casein ratio. Maubois and Lorient [24] calculated NCFs for different ratios of whey protein to casein (Table 4) and found that (1) the factors were in a very narrow range and (2) they were very close to the factor of 6.38 traditionally used.

Whey protein-to-casein ratio	NCF for infant formula
20:80	6.370
30:70	6.375
50:50	6.385
60:40	6.390

Table 4. Calculation of NCF for milk-based infant formula depending on the whey protein-to-casein ratio as reported by Maubois and Lorient [24]

4.2.2. Effect of whey protein profile on the NCF for milk-based infant formula

Whey protein can be obtained through different processes that can affect the protein profile of the finished product. To IDF's knowledge, no data has been reported to date on determining the effect of the whey protein profile on the NCF for infant formula. Hence, using the NCFs reported by van Boekel and Ribadeau-Dumas [8] and the typical percentage of protein in milk, the percentage of protein in whey (i.e. acid whey) was calculated. Sweet whey contains casein glycomacropeptide (cGMP), the cleavage product of κ-casein by chymosin (an enzyme contained in rennet). cGMP makes up approximately 20–25% of total sweet whey protein [37]. The percentage of protein in sweet whey was calculated using a figure of 20% (Table 5).

Product / protein	NCF (with carbs)	% Protein in milk	% Protein in whey fraction	% Protein in sweet whey
α_{s1}-Casein	6.36	30.3	-	-
α_{s2}-Casein	6.29	7.9	-	-
β-Casein	6.37	28.2	-	-
κ-Casein	6.35	10	-	-
γ-Casein	6.34	2.4	-	-
β-Lactoglobulin	6.29	9.7	54.5	43.6
α-Lactalbumin	6.25	3.6	20.2	16.2
Serum albumin	6.07	1.2	6.7	5.4
Proteose peptones	6.55	0.9	5.1	4.0
Immunoglobulins	6.20	2.4	13.5	10.8
cGMP	7.35	-	-	20.0
Milk protein	6.36	-	-	-
Isoelectric (acid) casein	6.36	-	-	-
Rennet whey proteins	6.41	-	-	-

Table 5. Nitrogen conversion factors for various milk proteins, according to van Boekel and Ribadeau-Dumas [8], and calculated percentages of whey proteins in the whey fraction and in sweet whey

cGMP has an NCF of 7.35 if carbohydrates are included and 6.73 when they are not [7]. This is much higher than the NCF for the other major milk proteins. Thus, the presence or absence of cGMP could be expected to affect the whey NCF.

Whey can also be processed (i.e. by cGMP removal, α-lactalbumin enrichment, *etc.*) to modulate its nutritional and/or biological properties. Table 6 shows the theoretical NCF values for whey protein (NCFw, shaded row) as calculated for different cGMP, α-lactalbumin and β-lactoglobulin contents using the relative concentrations of each protein and their corresponding NCF (NCFp). The lower part of Table 6 shows the NCFs for infant formulas calculated using different ratios of whey protein to casein (wp/c).

Table 6 shows that the NCF for infant formula made from different whey sources can range from 6.30 to 6.50. A value of 6.30–6.34 is applicable when acid whey protein or native whey protein is used (column 7; 0% cGMP), and 6.38-6.43 when sweet whey protein is used (column 3; 20% cGMP). The current NCF of 6.38 is very close to the mean (6.39) and equal to the median of the values (Figure 1). The potential heterogeneity in whey protein could cause the values to deviate to a maximum of only –1.2% and +1.9%, which means that the current factor of 6.38 can be applied to all milk-based infant formulas.

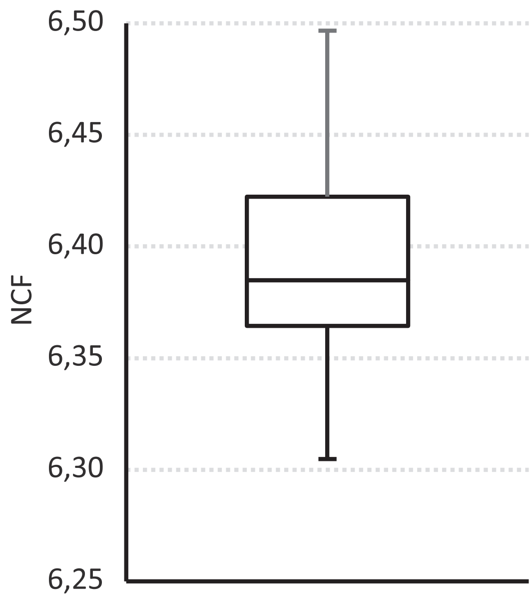

Figure 1. Box-plot of theoretical NCF values for infant formulas calculated in Table 6. The boxes represent the 1st, 2nd and 3rd quartiles and the whiskers indicate the minimum and maximum values

Protein	NCFp	Variation in [cGMP]					α-Lactalbumin enrichment				β-Lactoglobulin depletion			
		20%	15%	10%	5%	0%	1×	2×	3×	4×	1×	2×	3×	4×
β-Lactoglobulin	6.29	43.6	46.3	49.0	51.8	54.5	43.6	35.2	26.8	18.3	43.6	21.8	14.5	10.9
α-Lactalbumin	6.25	16.2	17.2	18.2	19.2	20.2	16.2	32.4	48.5	64.7	16.2	22.4	24.5	25.6
Bovine serum albumin	6.07	5.4	5.7	6.1	6.4	6.7	5.4	4.4	3.3	2.3	5.4	7.5	8.2	8.5
Proteose peptone	6.55	4.0	4.3	4.6	4.8	5.1	4.0	3.3	2.5	1.7	4.0	5.6	6.1	6.4
Immunoglobulins	6.2	10.8	11.5	12.1	12.8	13.5	10.8	8.7	6.6	4.5	10.8	15.0	16.3	17.0
cGMP	7.35	20.0	15.0	10.0	5.0	0.0	20.0	16.1	12.3	8.4	20.0	27.7	30.3	31.6
	NCFw	6.48	6.43	6.38	6.32	6.27	6.48	6.44	6.39	6.35	6.48	6.56	6.58	6.60
Product	wp/c	NCFw	NCFw	NCFw	NCFw	NCFw	NCFw	NCFw	NCFw	NCFw	NCFw	NCFw	NCFw	NCFw
Infant formula	20/80	6.38	6.37	6.36	6.35	6.34	6.38	6.38	6.37	6.36	6.38	6.40	6.40	6.41
Infant formula	30/70	6.40	6.38	6.36	6.35	6.33	6.40	6.38	6.37	6.36	6.40	6.42	6.43	6.43
Infant formula	50/50	6.42	6.40	6.37	6.34	6.31	6.42	6.40	6.38	6.35	6.42	6.46	6.47	6.48
Infant formula	60/40	6.43	6.40	6.37	6.34	6.30	6.43	6.41	6.38	6.35	6.43	6.48	6.49	6.50

Table 6. Nitrogen conversion factors (NCFw) for different theoretical whey protein profiles

NCFw were calculated based on the relative amount of the major proteins and their individual NCFs (NCFp). Values were computed for various theoretical cGMP concentrations, one to four-fold α-lactalbumin enrichment (based on the α-lactalbumin value in sweet whey containing 20% cGMP), and one to four-fold β-lactoglobulin depletion (based on the β-lactoglobulin value in sweet whey). Calculated NCFs for infant formulas, based on different ratios of whey protein to casein (NCF_{casein} = 6.36), are shown in the lower part of the Table. The lowest and highest values are highlighted

5
NUTRITION AND SUSTAINABILITY

A quarter of a century ago it was already being emphasized that the determination of protein is important in terms of nutrition and sustainability [14]. It is now increasingly recognized that providing nutritional security to a future population of between nine and ten billion people and ensuring sustainability of the planet's resources are two of the most significant global challenges.

FAO and Biodiversity International define sustainable diets as [38]:

… those diets with low environmental impacts which contribute to food and nutritional security and to healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy; while optimizing natural and human resources.

There is growing interest in the complex relationship between nutrition and environmental sustainability [39–43] and this relationship is a significant feature of the United Nations Sustainable Development Goals [44].

Hence, within this context it is important that appropriate scientifically valid methods are used to determine both the protein content and protein quality of foods. The use of NCFs for determining the protein content has been discussed in previous sections. An in-depth discussion of the determination of protein quality is beyond the scope of this Bulletin, but a brief overview is given in Appendix 2.

6 CONCLUSIONS

On the basis of a review of the scientific literature, and consideration of additional calculations presented in this Bulletin, it can be concluded that:

- The overwhelming consensus of scientific studies is that specific NCFs for specific foods should be used.
- For both dairy protein and soy protein, scientific publications based on experimental and/or theoretical analysis of NCFs consistently demonstrate that use of an NCF of 6.25 is incorrect and scientifically flawed.
- For soy products in general, the scientific literature reports NCFs in the range 5.6–5.8. The only value quoted higher than this range (6.30, for soy flour) was obtained through erroneous exclusion of nitrogen content from the amides contained in asparagine and glutamine.
- For soy protein isolates, data reported in the scientific literature indicate that the NCFs for these products (range 5.63–5.85; mean 5.73) are not substantially different from those for other soy products.
- For soy hydrolysates, the limited data reported in the scientific literature indicate that the NCFs for these products (range 5.56–5.59; mean 5.58) are similar to those for other soy products.
- Allowing for wide variation in the ratio of 11 to 7S proteins from different soy cultivars, the calculated NCF for soy-based infant formulas ranges from 5.69 to 5.79 (mean 5.74). This mean is very close to the value of 5.71 stated in the Codex Standard for Infant Formula [2] as applicable to soy-based infant formula.
- For milk-based infant formulas, allowing for (1) different ratios of whey protein to casein in the final product and (2) wide variation in whey protein composition as a result of different manufacturing processes, the calculated NCF ranges from 6.30 to 6.50 (mean 6.39). This mean is very close to the value of 6.38 stated in the Codex Standard for Infant Formula [2] as applicable for milk-based infant formula.

- The value of the NCF determined depends on whether or not:
  a) Glycosylated and/or phosphorylated prosthetic groups are included (excluding the prosthetic groups results in lower values for the NCF). It can be argued that these prosthetic groups are to be considered as constituent parts of the protein, because:
     - They are covalently bound to the amino acid backbone;
     - They have nutritional, physiological and technological functions.
  b) NPN is considered. An argument in favour of accounting for NPN when establishing NCFs is that the NPN fraction can contain substantial proportions of free amino acids and peptides.

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APPENDICES

APPENDIX 1 REFERENCES TO NITROGEN CONVERSION FACTORS WITHIN CODEX STANDARDS

Standard	Codex Committee	Title	Section	Relevant provision
CODEX STAN 1-1985	CCFL	General Standard for the Labelling of Prepack-aged Foods	4.2.3.1	Class name ”Milk Protein”. Milk products containing a minimum of 50% of milk protein (m/m) in dry matter*. *Calculation of milk protein content: **Kjeldahl nitrogen × 6.38**
CODEX STAN 72-1981	CCFNSDU	Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants	3.1.3 (a) Protein, Footnote 2	For the purpose of this standard, the calculation of the protein content of the final product prepared ready for consumption should be based on **N** × **6.25**, unless a scientific justification is provided for the use of a different conversion factor for a particular product. The protein levels set in this standard are based on a nitrogen conversion factor of 6.25. The value of **6.38** is generally established as a specific factor appropriate for conversion of nitrogen to protein in other milk products, and the value of **5.71** as a specific factor for conversion of nitrogen to protein in other soy products.
CODEX STAN 175-1989	CCVP	Codex General Standard for Soy Protein Products	2. Description	Soy Protein Products (SPP) covered by this standard are food products produced by the reduction or removal from soybeans of certain of the major non-protein constituents (water, oil, carbohydrates) in a manner to achieve a protein **(N** × **6.25**) content of:
			3.2.2	Crude protein (**N 6.25**) shall be: – in the case of SPF, 50% or more and less than 65% – in the case of SPC, 65% or more and less than 90% – in the case of SPI, 90% or more on a dry weight basis excluding added vitamins, minerals, amino acids and food additives.

Standard	Codex Committee	Title	Section	Relevant provision
CODEX STAN 234-1999	CCMAS	Recommended Methods of Analysis and Sampling		Soy protein products – Protein - AOAC 955.04D (**using factor 6.25**) - Titrimetry , Kjeldahl digestion Infant formula - Crude protein[1] - ISO 8968-1 | IDF 20-1 - Titrimetry (Kjeldahl) 1) Determination of Crude Protein *The calculation of the protein content of infant formulas prepared ready for consumption may be based on **N × 6.25**, unless a scientific justification is provided for the use of a different conversion factor for a particular product. The value of **6.38** is generally established as a specific factor appropriate for conversion of nitrogen to protein in other milk products, and the value of **5.71** as a specific factor for conversion of nitrogen to protein in other soy products* Edible casein products - Milk Protein (**total N × 6.38** in dry matter) - ISO 8968-1 | IDF 20-1 - Titrimetry, Kjeldahl Whey powders - Milk protein (**total N × 6.38**) - ISO 8968-1 | IDF 20-1 Titrimetry (Kjeldahl) Non-fermented soybean products - Protein content - NMKL 6 or AACCI 46-16.01 or AOAC 988.05 or AOCS Bc 4-91 or AOCS Ba 4d-90 (**Nitrogen factor 5.71**) - Titrimetry, Kjeldahl digestion Tempe - Protein content - NMKL 6 or AOAC 988.05 or AACCI 46-16.01 (**Nitrogen factor 5.71**) - Titrimetry, Kjeldahl digestion
CODEX STAN 243-2003	CCMMP	Codex Standard for Fermented Milks	Note (a) to table in section 3.3	Protein content is **6.38 multiplied by the total Kjeldahl nitrogen** determined
CODEX STAN 289-1995	CCMMP	Codex Standard for Whey Powders	Note (b) to table in section 3.3	Protein content is **6.38 multiplied by the total Kjeldahl nitrogen** determined

Standard	Codex Committee	Title	Section	Relevant provision
CODEX STAN 290-1995	CCMMP	Standard for Edible Casein Products	Note (a) to table in section 3.3	Protein content is **6.38 multiplied by the total Kjeldahl nitrogen** determined
CODEX STAN 298R-2009	CCASIA	Regional Standard for Fermented Soybean Paste (Asia)	Note 2 to Table in section 3.2	The **nitrogen conversion factor of 5.71** should be used.
CODEX STAN 313R-2013 Amendment 2015	CCASIA	Regional Standard for Tempe (Asia)	8 – Methods of sampling and analysis	Protein Content: NMKL 6, 2004 or AOAC 988.05 or AACCI 46-16.01 (**Nitrogen factor 5.71**)
REP15/ASIA APPENDIX IV	CCASIA	Draft Regional Standard for Non-Fermented Soybean Products (Step 8)	9.1.2 Determination of Protein Content	According NMKL 6, 2004 or AACCI 46-16.01 or AOAC 988.05 or AOCS Bc 4-91 or AOCS Ba 4d-90,**nitrogen factors for non-fermented soybean products are 5.71**.

APPENDIX 2 PROTEIN QUALITY

Protein is an important nutrient and must deliver all the indispensable amino acids in the correct balance for growth and maintenance. The protein content of a food is not the only criteria for adequate human nutrition; increasingly regulators [45], the food industry and health care professionals are recognizing the relevance of protein quality.

Protein quality refers to the ability of the amino acids in foods to adequately meet human requirements for indispensable amino acids. Amino acid requirements vary for specific age groups and physiological conditions [46]. The consequences of inadequate protein intake to meet indispensable amino acid requirements are well known and include stunted growth, increased susceptibility to infection, suboptimal muscle capacity and diminished mental performance (from retardation to apathy). Precise assessment of the ability of a dietary protein source to match the body's needs for individual amino acids allows better use of an increasingly scarce resource [47].

What are PDCAAS and DIAAS?

The Protein Digestibility Corrected Amino Acid Score (PDCAAS) is a simple and widely used methodology for evaluating protein quality. PDCAAS is derived from the ratio between the first limiting amino acid in the protein and its corresponding value from the amino acid reference pattern, and corrected for true faecal nitrogen (N) digestibility.

The PDCAAS is generally acceptable for the routine evaluation of the digestibility of protein from mixed human diets containing high quality protein sources. However, it could be inappropriate for evaluating the protein quality of foods that make up a major proportion of the diet, for example infant formula, enteral products, or novel or supplementary foods that contain anti-nutritional factors [47].

Furthermore, the PDCAAS has certain limitations [48-50]:

- PDCAAS values are truncated to 100%, or 1, which limits high quality proteins relative to poorer quality proteins and fails to recognize the advantages of surplus amino acids in complementing poorer quality proteins in mixtures. Truncation removes any nutritional differences between high protein foods such as milk and soy, although actual concentrations of important dietary indispensable amino acids, which may be limiting in some diets, are higher in milk than in soy. This could be recognized by giving individual protein sources an amino acid score of > 1 (or > 100).

- Faecal N digestibility likely overestimates the delivery of dietary amino acids to the body.

- Anti-nutritional factors in plant proteins or processed foods may lead to higher endogenous amino acid losses. Thus, PDCAAS may inappropriately reflect high scores.

- The amino acid reference pattern used is based on minimum requirements for growth and maintenance using the pattern for 2 to 5 year old children and does not reflect optimal intake.

Hence, the recent FAO Expert Consultation *"Dietary protein quality evaluation in human nutrition"* recommends a new, advanced method, the Digestible Indispensable Amino Acid Score (DIAAS) for assessing the quality of dietary proteins [47]:

$$\text{DIAAS} = \frac{\text{mg of digestible dietary indispensable amino acid in 1 g of the dietary protein}}{\text{mg of the same dietary indispensable amino acid in 1 g of the reference protein}}$$

FAO are convening expert groups to review research needs and develop a programme of work that will address the above questions and create the data needed to gain formal FAO endorsement of the DIAAS method.

EVALUATION OF NITROGEN CONVERSION FACTORS FOR DAIRY AND SOY

ABSTRACT

This Bulletin reviews the scientific literature pertaining to nitrogen conversion factors (NCFs) for dairy products and soy products. It presents substantial scientific evidence that supports the use of a specific NCF of 6.38 for milk protein and of 5.71 for soy protein, rather than a single inaccurate factor of 6.25.

Keywords: *nitrogen conversion factor, protein, milk, dairy, soy, soy protein isolates, soy hydrolysates, infant formula, Kjeldahl, whey protein profile, nutrition, sustainability, protein quality, Codex Alimentarius, FAO*

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  - Page number or number of pages, and date.

Example: 1 Singh, H. & Creamer, L.K. Aggregation & dissociation of milk protein complexes in heated reconstituted skim milks. J. Food Sci. 56:238-246 (1991).

Example: 2 Walstra, P. The role of proteins in the stabilization of emulsions. In: G.O. Phillips, D.J. Wedlock & P.A. William (Editors), Gums & Stabilizers in the Food Industry - 4. IRL Press, Oxford (1988).

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ANNEX 1

IDF CONVENTIONS ON SPELLING AND EDITING

In the case of native English speakers the author's national conventions (British, American etc.) are respected for spelling, grammar etc. but errors will be corrected and explanation given where confusion might arise, for example, in the case of units with differing values (gallon) or words with significantly different meanings (billion).

"	Usually double quotes and not single quotes
? !	Half-space before and after question marks, and exclamation marks
±	Half-space before and after
micr<u>oo</u>rganisms	Without a hyphen
Infra-red	With a hyphen
et al.	Not underlined nor italic
e.g., i.e.,...	Spelled out in English - for example, that is
lit<u>re</u>	Not liter unless the author is American
ml, mg,...	Space between number and ml, mg,...
skimmilk	One word if adjective, two words if substantive
sulfuric, sulfite, sulfate	Not sulphuric, sulphite, sulphate (as agreed by IUPAC)
AOAC <u>INTERNATIONAL</u>	Not AOAC<u>I</u>
progra<u>mme</u>	Not program unless a) author is American or b) computer program
milk and milk product	rather than "milk and dairy product" - Normally some latitude can be allowed in non scientific texts
-ize, -ization	Not -ise, -isation with a few exceptions
Decimal comma	in Standards (only) in both languages (as agreed by ISO)
No space between figure and % - i.e. 6%, etc.	
Milkfat	One word
USA, UK, GB	No stops
Figure	To be written out in full
1000-9000	No comma
10 000, etc.	No comma, but space
hours	Ø h
second	Ø s
litre	Ø l
<u>t</u>he Netherlands	

Where two or more authors are involved with a text, both names are given on one line, followed by their affiliations, as footnotes

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