UNIVERSIDAD SAN FRANCISCO DE QUITO

AMINO ACID COMPOSITION AND NITROGEN TO PROTEIN CONVERSION FACTORS FOR THREE LEGUMES AND TWO PSEUDO-CEREALS

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DEDICATORIA

A mi familia entera, especialmente a mis padres y hermana; en esta etapa final de mi vida universitaria, teniéndoles siempre presente por dedicarme su cuidado, afecto y respaldo a lo largo de mi carrera estudiantil. Con mucho cariño, el esfuerzo de este trabajo, para ustedes.

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RESUMEN

Cowpea (caupi), chocho y fréjol mung demostraron cómputos químicos de 64, 62, y 60, respectivamente; con los aminoácidos azufrados siendo los limitantes. Amaranto y quinua demostraron cómputos químicos de 74 y 90, respectivamente; con leucina siendo limitante en amaranto y valina en quinua. El contenido en histidina de todos los cultivos supera al requerimiento establecido por la FAO/WHO para niños. Los factores de conversión nitrógeno a proteína corregidos para el contenido en nitrógeno no proteico, son 6.01 para cowpea, 6.00 para chocho, 6.40 para fréjol mung, 5.52 para amaranto y 5.93 para la quinua.

Palabras claves: aminoácidos, nitrógeno, nitrógeno no proteico, proteína, leguminosa, pseudocereal, cowpea, caupi, chocho, lupino, fréjol mung, amaranto, quinua.

ABSTRACT

The results of a meta-analysis gave chemical scores for cowpea, lupine and mung bean of 64, 62, and 60, respectively, with the sulphur containing amino acids being limiting in these legumes. Amaranth and quinoa had chemical scores of 74 and 90, respectively, with leucine being limiting in amaranth and valine in quinoa. All species contain substantially more histidine than the FAO/WHO requirement for infants. The nitrogen to protein conversion factors corrected for nonprotein nitrogen content are: 6.01 for cowpea, 6.00 for lupine, 6.40 for mung bean, 5.52 for amaranth, and 5.93 for quinua.

Key words: amino acids, nitrogen, nonprotein nitrogen, protein, legume, pseudo-cereal, cowpea, lupine, mung bean, amaranth, quinoa.

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INTRODUCTION

Protein content of foods and feeds is calculated by multiplying the value determined for Kjeldahl nitrogen by a nitrogen to protein (N:P) conversion factor. Protein content is usually estimated by using 6.25 or 5.7 as default factors. The use of 6.25 as a nitrogen to protein conversion factor derives from early research on animal proteins which showed these to contain about 16% N (100 ÷ 16 = 6.25), while 5.7 derives from work on the gliadin and glutenin content of wheat as well as the nitrogen content of these protein fractions (Tkachuk 1969). Jones (1931) criticized the use of these two factors on the basis that they did not take into consideration the different nitrogen content of different protein sources and subsequently calculated nitrogen to protein factors ranging from 5.18 to 6.25 for various foodstuffs. Jones (1931) recognized that his calculations were also flawed because they did not take into consideration nonprotein nitrogen, but was unable to correct for this because of the limited information then available on such compounds. Foods may contain various other nitrogenous compounds including, but not limited to, nucleic acids, nitrate, nitrite, amines, vitamins, alkaloids, nitrogenous glycosides (Fujihara *et al.* 2008), amides and free amino acids (Ezeagu *et al.* 2002), and polyamines (Koziol 1992). The use of the default factor of 6.25 for foods containing appreciable amounts of these compounds will result in an overestimation of their protein content.

An accurate estimation of protein content is essential in nutritional research and in the formulation of foods and feedstuffs. In animal nutrition, protein excess as well as deficit can be detrimental. An excess of protein in feedstuffs is both uneconomical and, in the case of the feces of monogastric animals, harmful to the environment (Mossé 1990). In human nutrition, the IDF (2006) emphasized the importance of specific nitrogen to protein conversion factors to ensure compliance with established protein contents in infant formulas in response to a suggestion by the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) that protein content be assessed using the default N:P factor of 6.25. For example, Boisen *et al.* (1986) showed that using this default conversion factor when replacing dietary protein from skim milk powder with protein from grass meal would result in a formulation with 25% less protein. A similar error will be introduced in the assessment of protein quality by standard methods such as Protein Efficiency Ratio (PER), Biological Value (BV), and Net Protein Utilization (NPU), which

all require diets to be formulated with determined protein contents (Brody 1994). In such studies, the use of N:P conversion factors less than 6.25 will result in an underestimation of protein quality while values in excess in 6.25 will result in overestimation.

Of equal importance to protein content is protein quality, specifically as regards the profile of the essential amino acids and the identification of the limiting essential amino acid, which influences the chemical score of the protein (Brody 1994).

Legumes, cereals, and pseudo-cereals represent important sources of protein in human and animal nutrition. Annual production of quinoa is estimated at 48, 000 metric tonnes, with Bolivia producing 45% of the world's production, Peru 42%, the United States of America 6%, Canada 3%, and Ecuador 2%, with a minimum production in Europe (http://campocoop.cl/docs/ProductionQuinoa.). World lupine production for human and animal consumption in 2004 was estimated at one million metric tonnes: Australia produced 78% of this total (780,000 tonnes, primarily *Lupinus angustifolius*), 25 European countries produced 15% (220,000 tonnes) and the remainder produced in Africa, Russia and South America. France and South Africa produce white lupine, *Lupinus albus*, Central Europe produces the yellow lupine, *Lupinus luteus,* while South America primarily produces the bitter pearl lupine, *Lupinus mutabilis* (http://www.ifi-online.com/Tmpl_Article_Overview.asp).

World production of mung bean in 2005 was 122,882 million metric tones (MT), with China producing 100,214 (MT), Nigeria producing 3,025 (MT), and Uganda 2,604 MT (http://www.pcarrd.dost.gov.ph/commodities/velero/index.php). In 1997, world cowpea production was estimated at three million metric tonnes, with West and Central Africa accounting for 64% of this production (http://www.hort.purdue.edu/newcrop.html). There is little data on the world production and consumption of amaranth. The principal producer is China, with 150,000 hectares under cultivation, followed by India and Peru with 1800 hectares, Mexico with 900 hectares, and finally the United States of America with 500 hectares (http://www.cofecyt.mincyt.gov.ar.pdf).

The purpose of this study was to present a review of published information concerning the amino acid profiles of lupine (*Lupinus mutabilis* L.), mung bean (*Vigna radiata* (L.)

Wilcz.), cowpea (*Vigna unguiculata* (L.) Walp.), amaranth (*Amaranthus caudatus* L.), and quinoa (*Chenopodium quinoa* Willd.), and to combine these data with estimations of total and nonprotein nitrogen to calculate specific N:P factors for these species.

MATERIALS AND METHODS

Pseudo-cereal and legume seeds were obtained from the local market for analysis of total and nonprotein nitrogen content. Chemicals were provided by the Department of Food Engineering of the College of Agriculture, Foods and Nutrition, USFQ.

A meta-analysis approach was applied for compiling data on the amino acid compositions of these five species. Such data will thus reflect differences in crop varieties, climatic and edaphic factors, and will therefore represent a more comprehensive data set than that which would result from performing analyses on single samples from single sources. Where necessary, values reported for the amino acids by the various authors were converted to mg amino acid/gN. This obviates the need to convert data to a dry weight basis for although relative protein content may change with sample moisture content, the protein composition in terms of the constituent amino acid residues remains constant.

ANALYTICAL

Preparation of samples

Pseudo-cereal and legume seed were finely ground with a Straub Model 4E Grinding Mill to pass a 40 mesh screen (USA standard, 425 μm). Samples were stored in tightly sealed polypropylene bags until analysis.

Determination of total nitrogen

Total nitrogen content was determined in duplicate by the Kjeldahl method (AOAC 1990). Using a Mettler-Toledo balance (Model AB204-S), approximately 0.5 g of ground

sample was weighed into a Kjeldahl digestion flask to which was added 20 mL of analytical grade concentrated sulphuric acid (96.1% or 95.7%), a Kjeldahl digestion tablet, and boiling chips. The sample was placed on a Labconco Digestion Unit and digested for 3-4 hours, according to the sample being analyzed. Cooled samples were then distilled using a Büchi Model 320 distillation apparatus. The distillate was collected in 50 mL of standardized 0.1N H_2SO_4, then titrated with 0.1 N NaOH using methyl red as the indicator.

Determination of nonprotein nitrogen

The nonprotein nitrogen content (NPN) of the pseudo-cereal and legume seeds was determined in duplicate by method of Lees (1982). Using a Mettler-Toledo balance (Model AB204-S), 2.5 g of sample and 5 g of water were weighed into a centrifugation tube. To this mixture were added 1.25 g of 50% trichloroacetic acid and the contents of the tube mixed. Four repetitions were performed to achieve an extraction of 10 g of sample. The tubes were then centrifuged at 850×*g* for 15 minutes. *In toto*, 10 mL supernatant were collected from the four tubes and transferred to a Kjeldahl digestion tube for determination of nitrogen content according the Kjeldahl method described above.

Protein nitrogen

Protein nitrogen was calculated simply as the difference between total and nonprotein nitrogen:

$$\text{Protein N} = \text{Total N} - \text{Nonprotein N}$$

CALCULATIONS

Chemical Score

The chemical score was calculated according to Brody (1994) by comparing the essential amino acids of the protein in question to the amino acid scoring pattern reported by Harper (1981), using the formula $[(AA_x)\times 100] \div AA_e$, where "x" represents the essential amino acid in the protein in question and "e" the amino acid in the reference pattern. The chemical score is the minimal value and the corresponding amino acid is the limiting amino acid. Histidine was included as it has been shown to be essential for infants (Harper 1981).

N:P Conversion Factors

The values reported in the literature represent amino acids in their free base form. To reflect the state in which the amino acids occur in proteins the values for the individual amino acids were corrected for the water lost during the formation of peptide bonds. The correction factors for expressing the concentrations of each amino acid residue (AAres) in its anhydrous form were calculated by:

$$\text{AAres correction factor} = \frac{(\text{MWt AA}) - (\text{MWt } H_2O)}{(\text{MWt AA})}$$

Thus for glycine, the correction factor is:

$$(75.1 - 18) \div 75.1 = 0.76$$

The application of such correction factors to all amino acid residues introduces an insignificant error as the N and C terminal amino acids in a protein are not present in an anhydrous form. However, the relative number of end groups is small compared with the total number of amino acid residues in proteins and Tkachuk (1969) has estimated this error to be on the magnitude of 0.035%.

Amino acid nitrogen (AAN) was calculated by multiplying the anhydrous AAres content by a factor derived by dividing the molecular weight of the total number of nitrogen atoms present in amino acid by its anhydrous molecular weight.

$$\text{AAN factor} = [(N^o\ N)(\text{MWt N})] \div [(\text{MWt AA}) - (\text{MWt } H_2O)]$$

Thus, for glycine the factor for determining amino acid nitrogen is determined as:

$$[(1)(14)] \div [75.1 - 18] = 0.25$$

and for arginine:

$$[(4)(14)] \div [174.2 - 18] = 0.36$$

Preliminary N:P conversion factors, uncorrected for nonprotein nitrogen content, can now be calculated as:

$$\text{Preliminary N:P conversion factor} = \Sigma AAres \div \Sigma AAN$$

The presence of nonprotein nitrogen will reduce the values of the N:P conversion calculated solely on the basis ΣAAres ÷ ΣAAN (Tkachuk 1969). The true N:P conversion factor can be calculated as:

$$\text{N:P conversion factor} = [(\Sigma AAres)(\text{Total N} - \text{Nonprotein N})] \div [(\Sigma AAN)(\text{Total N})]$$

RESULTS AND DISCUSSION

The total content of the essential amino acids in the five species analyzed exceeded the pattern established by the FAO/WHO (Harper 1981) and chemical scores ranged from 60 for mung bean to 90 for quinoa (Table 1). The sulphur-containing amino acids were limiting in cowpea, lupine, and mung bean, with respective chemical scores of 64, 62, and 60, consistent with what would be expected for legumes (Sosulski and Holt 1980).

Table 1. Essential amino acid composition (mg AA/g N) and chemical scores.

	Cowpea[1]		Lupine[2]		Mung bean[3]		Amaranth[4]		Quinoa[5]		FAO/WHO[6]
	AA	Score	AA	Score	AA	Score	AA	Score	AA	Score	AA Pattern
Histidine	207	230	175	194	191	212	145	161	188	209	90
Isoleucine	342	137	274	110	278	111	247	99	269	108	250
Leucine	494	112	449	102	478	109	326	74	411	93	440
Lysine	443	130	363	107	427	126	377	111	367	108	340
Methionine + Cystine	141	64	136	62	131	60	237	108	250	114	220
Phenylalanine + Tyrosine	542	143	469	123	586	154	423	111	471	124	380
Threonine	250	100	238	95	225	90	247	99	232	93	250
Tryptophan	68	113	67	112	70	117	77	128	69	115	60
Valine	310	100	269	87	320	103	270	87	280	90	310
Total	2797	——	2440	——	2706	——	2349	——	2537	——	2340
Chemical score		64		62		60		74		90	

[1] Madamba *et al.* (2006), Khan and Baker (1957), Lambot (2002), Amjad *et al.* (2006), Devarajan (2004), Vijayaraghavan and Srinivasan (1953), Elias *et al.* (1964), Phillips and Baker (1987), Rangel *et al*. (2004), FAO (1970).

[2] Feldheim (1998), Freire (1984), Hung (1993), Schoeneberger *et al.* (1982), Tapia (1997), Villacrés *et al.* (2003), FAO (1970).

[3] Badshah Khattak and Klopfenstein (1989), Bagchi *et al.* (1955), Vijayaraghavan and Srinivasan (1953), Bhatty *et al.* (2000), Khalil (2005), Mogotsi (2006), Wills *et al.* (1984), Lambot (2002), Mubarak (2005), Khader and Venkat Rao (1996), FAO (1970).

[4] Tapia (2000), PÍSAŘIKOVÁ *et al.*(2005), Saunders and Becker (1984), Gamel *et al* (2004), Mujica y Jacobsen (2006), FAO (1970)

[5] Wright *et al.* (2002), Koziol, (1992), Cusack (1984), Ruales *et al.* (1992), Tellería *et al.* (1978), Mujica and Jacobsen (2006), Fujihara *et al.* (2008), FAO (1970)

[6] Harper (1981). The scoring pattern reflects adequacy for young children, plus the histidine requirement for infant**s.**

Cereals are generally deficient in lysine (Fujihara *et al.* 2008), but this is not the case with the pseudo-cereals amaranth and quinoa. Leucine, with a chemical score of 74, was limiting in amaranth and valine, with a chemical score of 90, was limiting in quinoa. All species contained substantially more histidine than the FAO/WHO pattern, which makes them suitable protein sources for use in infant formulas. All species also contained substantially more of the aromatic amino acids phenylalanine and tyrosine.

With the exception of a deficiency in the sulphur containing amino acids, cowpea is more than adequate as regards the remaining essential amino acids, lupine adequate in all but threonine and valine, and mung bean in all but threonine. The lysine (280 mg lysine/gN) and tryptophan (34 mg tryptophan/gN) concentrations in lupine reported by Sosulski and Holt (1980) are substantially less than found in this study. This may be attributed to the fact that their study is based on the analysis of only one sample of an unidentified variety of lupine (Lupinus sp.) whereas the sample base for the current study is more ample. The values for mung bean closely approximate those of Sosulski and Holt (1980).

In comparison with other vegetable protein sources, amaranth and quinoa have the highest chemical scores (Table 2). Cowpea, lupine, and mung bean are similar to oats in chemical score and superior to wheat, maize, lima beans, and lentils. In amaranth, quinoa, cowpea, lupine, and mung bean, total protein content is as important as the chemical score and profiles of the essential amino acids are factors which should be taken into consideration when formulating foods and feedstuffs with these species.

Table 2. Comparison of chemical scores of different protein sources.

Protein source[1]	Chemical score[1]	Protein source[1]	Chemical score[1]
Egg	100	Rice	56
Quinoa	90	Peanuts	55
Amaranth	74	Soybeans	47
Cowpea	64	Wheat	43
Lupine	62	Maize	41
Mung bean	60	Lima beans	41
Oats	57	Lentils	31

[1] Data from Brody (1994), with the exception of quinoa, amaranth, cowpea, lupine, and mung bean.

Total nitrogen content ranged from 2.75% for amaranth to 6.85% for lupine (Table 3), well within published values. For example, the value for amaranth (2.75%) is close to the 2.56% reported by Fujihara *et al.* (2008). For quinoa, the value of 3.09% is higher than the 2.12% reported by Fujihara *et al.* (2008), but is still within the range of values expected for quinoa (Koziol, 1992). Sosulski and Holt (1980) reported values of 6.17% and 4.25% for lupine and mung bean, respectively, which compare favorably to the values obtained in this study, namely 6.85% and 4.05%, respectively.

Several problems are inherent to the determination of nitrogen to protein conversion factors. Firstly, few investigators have reported amino acid profiles for several varieties of one species sampled over multiple harvest periods. As it is tenuous to base a conversion factor on such limited analytical data a meta-analysis approach has been used in this study. Secondly, very few studies have included hydroxyproline, asparagine, and glutamine in the amino acid profiles (vanEtten *et al.* 1963; Tkachuk, 1969; Koziol 1992; Fujihara *et al.* 2008). Asparagine and glutamine, the amide derivatives of aspartic and glutamic acids, can represent important sources of amino acid nitrogen; for example Tkachuk (1969) reported 108 μM asparagine, 299 μM aspartic acid, 2172 μM glutamine, and 299 μM glutamic acid per gram of protein in Manitou wheat, and Fujihara *et al.* (2008) reported 263 mg asparagine, 319 mg aspartic acid, 484 mg glutamine, and 586 mg glutamic acid per gram of nitrogen on a dry weight basis in brown rice. The exclusion of these amino acids in the calculation of nitrogen to protein conversion factors is a serious omission.

Another source of error is the determination of nonprotein nitrogen (NPN). Bell (1963) published a comprehensive critical review of twenty methods then available and concluded that "The progress of studies on NPN fractions depends largely on the availability of reliable methods for the primary separation of protein from nonprotein nitrogen… even the simplest procedure for removing protein does not achieve separation purely on molecular size… sources of error inherent in different methods include the hydrolysis of nitrogen-containing polymers, the absorption of NPN compounds onto protein, and the anomalous behavior of some compounds with different protein precipitants." Of the methods investigated, minimum binding of NPN to proteins was found when proteins were precipitated with trichloroacetic acid, which also resulted in a better extraction of NPN than with other methods (Bell 1963).

Various authors have analyzed specific nonprotein nitrogen fractions. Amide N was analyzed separately in field peas (Holt & Sosulski 1979) and for grain legumes (Sosulski & Holt 1980) and then classified as "other nitrogen". Without a specific distinction between amide N and the carbamide N deriving from asparagine and glutamine (de Rham 1982), this procedure is questionable as it would attribute amino acid nitrogen to the nonprotein fraction, thus introducing an important error in the determination of nitrogen to protein conversion factors. Other special cases exist. For example, in the case of mushrooms and common Japanese vegetables with high nitrate concentrations, Fujihara *et al.* (1995, 2001) applied a modification of the Kjeldahl procedure for samples with high nitrate concentrations, as well as analytical methods designed to quantify nitrate N, ammonia N, and nucleic acid N. Given the chemistry of grain legumes and pseudo-cereals the determination of nonprotein nitrogen can be simplified by using the trichloroacetic acid method (Bell 1963; Lees 1982).

Total amino acid concentrations for lupine and mung bean were close to the values reported by Sosulski and Holt (1980), namely 5720 and 6240 mg/gN, respectively, *versus* 5914 and 6001 mg/gN (Table 3). Other values cannot be compared for lack of available data.

The nitrogen to protein conversion factors corrected for nonprotein nitrogen content are: 6.01 for cowpea, 6.00 for lupine, 6.40 for mung bean, 5.52 for amaranth, and 5.93 for quinoa (Table 3). These values are compared with those reported in the literature (Table 4). The values published by Sosulski and Holt (1980) for lupine and mung bean are low, and were supposedly calculated according to Tkachuk (1969). However, recalculating their data according to the method of Tkachuk (1969) gave values closer to those reported in this study. The nitrogen to protein conversion factors for amaranth and quinoa are higher than those reported by Fujihara *et al.* (2008), but they analyzed only one sample of each pseudo-cereal. No data was found with which to compare the nitrogen to protein conversion factor for cowpea.

Table 3. Amino acid composition and nitrogen distribution (mg AA/g N), total, nonprotein and protein nitrogen (%), and nitrogen to protein conversion factors.

	Cowpea[1]	Lupine[1]	Mung bean[1]	Amaranth[1]	Quinoa[1]
Cystine	57 ±31	73 ±21	44 ±14	105 ±29	106 ±36
Histidine	207 ±13	175 ±22	191 ±46	145 ±29	188 ±19
Isoleucine	342 ±158	274 ±23	278 ±63	247 ±67	269 ±61
Leucine	494 ±87	449 ±30	478 ±56	326 ±25	411 ±39
Lysine	443 ±35	363 ±47	427 ±78	377 ±54	367 ±40
Methionine	84 ±25	63 ±39	87 ±33	132 ±33	144 ±37
Phenylalanine	363 ±66	243 ±20	337 ±75	244 ±21	277 ±34
Threonine	250 ±40	238 ±10	225 ±46	247 ±56	232 ±35
Tryptophan	68 ±18	67 ±23	70 ±24	77 ±17	69 ±9
Tyrosine	179 ±43	226 ±71	249 ±204	179 ±95	194 ±36
Valine	310 ±39	269 ±32	320 ±58	270 ±18	280 ±39
Alanine	220 ±70	226 ±12	238 ±58	300 ±71	282 ±26
Arginine	541 ±241	542 ±55	372 ±91	559 ±141	493 ±77
Aspartic acid	634 ±126	603 ±124	783 ±89	486 ±125	461 ±31
Glutamic acid	921 ±237	1261 ±154	1051 ±223	924 ±150	816 ±51
Glycine	216 ±49	266 ±8	213 ±57	594 ±170	348 ±46
Proline	254 ±19	241 ±16	330 ±142	206 ±34	224 ±34
Serine	262 ±69	337 ±38	307 ±6	405 ±130	235 ±21
Total Essential Amino Acids	2797	2440	2706	2349	2537
Total Amino Acids	5845	5914	6001	5823	5398
AAres[2]	5049	5108	5182	4986	4642
AAN[3]	815	817	793	846	764
N:P Factor	6.20	6.25	6.54	5.89	6.08
% Total Nitrogen	3.88	6.85	4.05	2.75	3.09
% Nonprotein Nitrogen	0.12	0.27	0.08	0.17	0.08
% Protein Nitrogen	3.76	6.58	3.97	2.58	3.01
Corrected N:P Factor	6.01	6.00	6.40	5.52	5.93

[1] Data from same references as given for these species in Table 1, ± standard deviations of the means.

[2] Anhydrous weight of the amino acid residues (mgAA/gN).

[3] Nitrogen content of the anhydrous amino acid residues (mgAAN/gN).

Table 4. Comparison of nitrogen to protein conversion factors.

Reference	Lupine	Mung bean	Amaranth	Quinoa
This study	6.00	6.40	5.52	5.93
Sosulski and Holt (1980)	4.94	5.38	——	——
Sosulski and Holt (1980), recalculated[1]	5.98	6.02	——	——
Fujihara *et al.* (2008)	——	——	4.74	5.39

[1] Nitrogen to protein conversion factors recalculated according to the method of Tkachuk (1969).

CONCLUSION

Use of the accepted nitrogen to protein conversion factor of 5.7 for legumes (de Rham 1982) will underestimate the protein content of cowpea, lupine, and mung bean, while the use of the default value of 6.25 will overestimate the protein content of amaranth and quinoa. A more precise determination of the nitrogen to protein conversion factors will allow a more exact evaluation of protein quality through standard methods such as protein efficiency ratio, biological value or net protein utilization.

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