Received: 19 December 2022 | Revised: 24 March 2023 | Accepted: 2 April 2023

DOI: 10.1002/sfp2.1008

ORIGINAL ARTICLE

AOCS WILEY

How different amino acid scoring patterns recommended by FAO/WHO can affect the nutritional quality and protein claims of lentils

Amanda G. A. Sá[1] | **Jiayi Hang**[1] | **Laura Jardine**[2] | **Kirstin E. Bett**[2] | **James D. House**[1]

[1]Richardson Centre for Food Technology and Research, Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

[2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Correspondence
James D. House, Richardson Centre for Food Technology and Research, Department of Food and Human Nutritional Sciences, University of Manitoba, 196 Innovation Drive, Winnipeg, MB, Canada.
Email: james.house@umanitoba.ca

Funding information
BASF; Genome Canada, Grant/Award Number: LSP18-16302; Saskatchewan Ministry of Agriculture, Grant/Award Number: 20200026; Saskatchewan Pulse Growers, Grant/Award Number: BRE 1516; University of Manitoba; University of Saskatchewan; Western Grains Research Foundation, Grant/Award Number: GC1903

Abstract

As a nutritious pulse and protein source, lentils play an important role in the plant-based protein market. Pulses' nutritional quality is influenced by their protein content and amino acid composition. Recommended scoring patterns by FAO/WHO can estimate protein quality for dietary assessment, but different guidelines for protein content in food labeling exist in North America. This study determined the *in vitro* protein digestibility (IVPD) and amino acid score (AAS) for the protein quality assessment of lentils. The impact of different recommended amino acid scoring patterns by FAO/WHO (1991, 2013) on AAS and AAS corrected for *in vitro* protein digestibility (AAS-IVPDC) were evaluated. The impact of AAS-IVPDC for determining protein content claims for lentils using USA standards was also evaluated. Sulfur AA and tryptophan were the most limiting amino acids. From this work, estimates of lentil protein quality vary with different recommended amino acid scoring patterns. IVPD in lentils was 82.6%, while mean AAS-IVPDC values ranged from 37.5% to 64.0%. Regarding the protein content claims, if considering a similar interpretation to the protein digestibility–corrected amino acid score (PDCAAS) system (i.e., corrected content is ≥ 5.0 g per RACC), all lentil samples were considered a "good source of protein." However, if considering a similar interpretation to digestible indispensable amino acid score (DIAAS) system (i.e., corrected content is ≥ 5.0 g per RACC and a claim threshold of 75%), no samples met these protein claims due to the arbitrary cut-off. The criteria set for making protein content claims should be revised.

KEYWORDS
amino acid requirements, food protein, legume protein, protein digestibility, protein quality

INTRODUCTION

Plant-based protein is a fast-growing market worldwide due to its environmental, economic, health, and social benefits (Health Canada, 2019a). Lentils play an important role in the plant-based market as a nutritious pulse. In recent decades, Canada has been the leading country in lentil production and exportation. Compared to traditional animal-based proteins, lentils are more affordable and environmentally friendly. They are desired by vegans and vegetarians as a protein source and have high protein content, fibers, vitamins, and minerals, thus providing health-promoting benefits for the consumer (Health Canada, 2019a).

As reported by previous studies, lentil seed protein content can vary from 10.5% to 36.4% (Khazaei et al., 2019; Kumar et al., 2016). Regarding essential amino acids, like other leguminous seeds, lentils are

generally low in sulfur amino acids (methionine and cysteine) and tryptophan (Boye et al., 2012; Nosworthy & House, 2017) relative to cereals. On the other hand, they have a high lysine content, which is generally deficient in cereals. This is why combining cereals and lentils provide a complete indispensable amino acid profile, enhancing the resultant protein quality and nutritional value.

The plant-based protein market growth trend, alongside the high production yield of lentils in Canada, has amplified the focus on studying the protein and amino acid contents of lentils, with a particular emphasis on protein quality (protein digestibility, amino acid profile, and bioavailability for absorption). Protein quality evaluation determines the capacity of the food protein to satisfy the human body's metabolic demands for amino acids and nitrogen (Boye et al., 2012; Sá et al., 2019).

Currently in North America, the protein efficiency ratio (PER) and protein digestibility–corrected amino acid score (PDCAAS) are being used to assess the quality of dietary proteins. PER is a bioassay that measures a rat's body weight gain over 28 days as a function of their protein intake and is used in Canada's protein content claims regulations (CFIA, 2021b; Health Canada, 1981). PDCAAS is calculated by multiplying true fecal nitrogen digestibility (also a rat bioassay) by the amino acid score (FAO/WHO, 1991) and has been implemented in the United States as the required method for protein quality determination and evaluation of the eligibility of foods for protein content claim purposes targeted at children through adults (FAO/WHO, 1991). Sufficient evidence is now available to sustain an interim change in Canada's protein quality regulations to harmonize with those of the United States (PDCAAS) (Marinangeli & House, 2017).

In a 2013 report by the FAO Expert Committee on Protein Evaluation in Human Nutrition, the PDCAAS approach to assessing protein quality was criticized, and a new method was proposed: DIAAS, the digestible indispensable amino acid score. DIAAS addresses methodological PDCAAS concerns by advocating untruncated protein scores and using each amino acid's ileal digestibility coefficient to determine the true ileal digestibility of the indispensable amino acids present within the food mixture. In the case of processed foods and lysine, DIAAS uses the true ileal digestibility of reactive lysine. DIAAS currently relies on using animals (ileal cannulated pigs) to assess protein quality (FAO/WHO, 2013). Although DIAAS is the most recently developed method for measuring protein quality and has been recommended in this 2013 FAO/WHO report, it is not yet used for regulatory purposes for the substantiation of protein content claims.

At this time, all three methods for protein quality evaluation involve animal experimentation to quantify protein digestibility, a practice society is attempting to move away from where possible (Langley et al., 2007). As an additional alternative to bioassay-derived digestibility, *in vitro* methods for the approximation of PDCAAS and DIAAS measurements could provide a low-cost and fast approach with potentially satisfactory sensitivity for regulatory needs and promising impacts on protein nutrition guidelines (Marinangeli & House, 2017).

Humans require adequate nitrogen intake (i.e., essential amino acids from available sources), which can vary with age, sex, weight, physiological conditions, and health state (FAO/WHO, 1991). Therefore, the joint FAO/WHO Expert Consultation on Protein Quality Evaluation has emerged to guide the essential amino acid requirements and establish recommended scoring patterns to calculate protein quality for dietary assessment (FAO/WHO, 1991, 2007, 2013). Scoring patterns for specific age categories (i.e., infants, children, adults) are regularly applied for protein quality evaluation of a dietary protein source. Current U.S. regulations stipulate that the amino acid requirements of pre-school children (aged 2–5 years) be used as the reference scoring pattern to determine the PDCAAS of foods (FAO/WHO, 1991). With the FAO/WHO (2013) recommendation to switch to DIAAS methods for protein quality evaluation, new age categories for amino acid scoring patterns were also proposed: (a) infants (birth to 6 months), (b) young children (6 months to 3 years), and (c) older children, adolescents, and adults (FAO/WHO, 2013).

Qualifying food sources for protein content claims is important for the industry sector and directs consumers toward dietary patterns that align with national dietary guidance. In the United States, PDCAAS and the crude protein content in the Reference Amount Customarily Consumed (RACC) are used to calculate the "Corrected Protein Content in RACC" (FDA, 2015). Foods qualify as a 'good source of protein' if the corrected content is 5.0–9.9 g per RACC and as an 'excellent source of protein' if it is ≥ 10 g per RACC. In Canada, foods qualify for 'source' and 'excellent source' protein claims if their protein ratings are ≥ 20 and ≥ 40, respectively (PER multiplied by the protein amount (g) in serving). The protein amount found in a reasonable daily intake of a given food is provided by Schedule K in the Canadian Food and Drug Regulations (CFIA, 2021a). The FAO/WHO (2013) recommendation states that DIAAS has a claim threshold of 75%. This means that when DIAAS is less than 75% and corrected protein content is lower than 5.0 g per RACC, the food is not considered a 'good source' of protein. To meet the claim of a 'good source' of protein, the corrected protein content must be 5.0–9.9 g per RACC, and DIAAS > 75%. To be considered an 'excellent source' of protein, the corrected protein content must be ≥ 10 g per RACC and DIAAS ≥ 100% (FAO/WHO, 2013). It is presently unclear to the authors of this study why DIAAS has a claim threshold of 75% for food to be considered a 'good source' of protein.

What are the implications of adopting the FAO/WHO Expert Consultation on Protein Quality Evaluation (2013) recommendations for protein quality evaluation on plant and plant-based foods labeling? To the best of the

authors' knowledge, no investigations have been published to explore this question. However, a comprehensive study on protein content has been undertaken on a lentil diversity panel grown in multiple locations in western Canada (Hang et al., 2022). As a follow-up to that study, this paper examined the impacts of policy change on protein content claims in whole lentils. The authors hypothesize that (a) the estimated protein quality of lentils can be affected by changing the amino acid scoring pattern recommended by FAO/WHO (2013), and (b) protein content claims for lentils can be negatively impacted if using the interpretation of a cut-off threshold of 75% (similar interpretation of DIAAS system). The main goal of this paper is to evaluate how different amino acid scoring patterns impact the protein quality and content claims in lentils. This work also introduces *in vitro* protein digestibility and amino acid score corrected for *in vitro* protein digestibility (AAS-IVPDC). Given the sheer number of samples derived from crop breeding programs, it is not feasible to conduct animal bioassays to ascertain variability in protein and amino acid digestibility coefficients within a single crop due to genetic, environmental, or processing factors. Different recommended age-category amino acid scoring patterns positioned by FAO/WHO (1991, 2013) were used to determine their impact on the nutritional quality and allowable protein content claims for lentils.

MATERIALS AND METHODS

Samples

Lentil samples ($n = 1290$) were secured from the Crop Development Centre (CDC) at the University of Saskatchewan. These samples consisted of 324 lentil genotypes from a panel of diverse varieties originating from all over the world. They were grown for two consecutive years (2016 and 2017) in two locations in Saskatchewan, Canada (Rosthern and Sutherland). The raw whole lentil samples were ground (≤ 0.75 mm) and stored in a −20°C freezer until further analyses.

Protein content

The Dumas combustion method determined the lentil protein contents (method 990.03, AOAC International, 2012). The nitrogen analysis, conducted by Central Testing Labs (Winnipeg, MB, Canada) was used to measure nitrogen and crude protein ($N \times 6.25$) through a standard nitrogen conversion factor (Mariotti et al., 2008).

Amino acid composition

The indispensable amino acid contents of the lentil samples ($n = 1290$), such as histidine (HIS), isoleucine (ILE), leucine (LEU), lysine (LYS), threonine (THR), tryptophan (TRP), valine (VAL), methionine (MET) + cysteine (CYS), and phenylalanine (PHE) + tyrosine (TYR); and the dispensable amino acids: alanine (ALA), arginine (ARG), aspartic acid (ASP), glutamic acid (GLU), glycine (GLY), serine (SER), and proline (PRO), were previously predicted using near-infrared reflectance spectroscopy (NIR) equipment (models DA 7250 and FT 9700, PerkinElmer Health Sciences Canada Inc., Winnipeg, Canada), as reported by Hang et al. (2022). The predicted amino acid composition was used to calculate the amino acid score, further described in Section 2.4, see Figure 1a–i.

For NIR calibration, a subset of 360 lentil samples was assayed using wet chemistry to determine amino acid composition. Separate hydrolysis methods were required to determine the complete amino acid profile due to different amino acid stabilities under hydrolysis conditions. The regular amino acids were analyzed using the AOAC method 982.30. The performic acid oxidized hydrolysis procedure (method 985.28) (AOAC International, 2012) was used to determine methionine and cysteine profiles. Sample analysis was conducted using a Shimadzu Nexera ultra-high performance liquid chromatography (UHPLC) system (Kyoto, Japan) equipped with a Waters AccQ C18 column (100 mm × 2.1 mm, 1.7 μm). The column oven temperature was 51°C for regular amino acids and 40 and 60°C for cysteine and methionine, respectively. The regular amino acids were determined by UV detection at 260 nm and the run time was 17 min. For sulfur amino acids, the detection was by fluorescence with excitation at 266 nm and emission at 473 nm, and the run time was 30 min for each sulfur amino acid. The Lab Solutions software (Shimadzu, Kyoto, Japan) was used to process data from the UHPLC. Tryptophan was determined using alkaline hydrolysis, following the ISO 13904:2005 method (ISO, 2005). The sample was injected into a Phenomenex Luna C18 column (250 mm × 4.6 mm, 3 μm) with a flow rate of 1 mL/min. The running time by reversed-phase UPLC was 34 min. Specific fluorescence detection was applied using an excitation wavelength of 280 nm and an emission wavelength of 356 nm. The amino acid composition ($n = 40$) from the wet chemistry method was used to estimate the amino acid score used on AAS-IVPDC, further described in Section 2.6.

Amino acid score

The amino acid compositions of the lentil samples were used to estimate the Amino Acid Score (AAS) as [mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern] × 100 (FAO/WHO, 1991). Different age-categories scoring patterns were used:

(a) pre-school children (2–5 years) (FAO/WHO, 1991); (b) infants (birth to 6 months), (c) young children (6 months to 3 years), and (d) older children, adolescents, and adults (FAO/WHO, 2013). The lowest AAS calculated reflects the first-limiting amino acid in the protein source and is used to establish the overall score (FAO/WHO, 1991).

In vitro protein digestibility (IVPD)

The method by Hsu et al. (1977) with minor modifications (Tinus et al., 2012) was used to determine the IVPD of a subset of lentil samples (a total of 40 samples, randomly picked from each quartile of protein content). The protein suspension (6.25 mg protein/mL distilled water) was adjusted to pH 8.0 with 0.1 N NaOH or 0.1 M HCl while stirring at 37°C. An enzyme mix containing 1.6 mg of trypsin (porcine pancreatic trypsin type IX-S, 13,000–20,000 Na-benzoyl-L-arginine ethyl ester (BAEE) units/mg protein, T0303, Sigma-Aldrich, St. Louis, MO, USA), 3.1 mg of α-chymotrypsin (bovine pancreatic chymotrypsin type II, ≥ 40 *N*-Benzoyl-L-Tyrosine Ethyl Ester (BTEE) units/mg protein, C4129, Sigma-Aldrich, St. Louis, MO, USA), and 1.3 mg of peptidase (Protease from *Streptomyces griseus* Type XIV, ≥ 3.5 units protease/mg solid, P5147, Sigma-Aldrich, St. Louis, MO, USA) per mL was maintained in an ice-bath and adjusted to pH 8.0. The enzymatic solution was added to the protein solution at a 1:10 v/v ratio and stirred at 37°C. The amino acid carboxyl groups released from the proteolytic enzymes' protein chain induced a rapid decrease in the pH value. The pH mixture was measured after 10 min using a pH meter. IVPD as a percentage of digestible protein was estimated according to pH variation after 10 min (ΔpH_{10min}), as shown in Equation (1).

$$IVPD\,(\%) = 65.66 + 18.10 \times \Delta pH_{10min} \quad (1)$$

Amino acid score corrected for *in vitro* protein digestibility (AAS-IVPDC)

The lowest AAS is used for the calculation of AAS-IVPDC as a product of the AAS and IVPD values for each sample evaluated (Nosworthy et al., 2018b), using FAO/WHO (1991) scoring patterns for pre-school children (2–5 years), and FAO/WHO (2013) scoring patterns for infants (birth to 6 months), young children (6 months to 3 years), and older children, adolescents, and adults.

Statistical analysis

The experimental data statistical analysis was conducted using the Statistica® software (v.13.5, Statsoft Inc., Tulsa, USA), adopting a confidence level of 95%. The results of IVPD and different AAS-IVPDC of the subset of lentil samples were compared via one-way ANOVA (Analysis of Variance) with Tukey's selected as the post hoc test. These analyses were performed in triplicate, and results are expressed as the average ± standard deviation of replicated samples. Correlation analysis between protein content and *in vitro* protein digestibility was evaluated using GraphPad® Prism software (v.9.0, GraphPad Inc., California, USA), using Pearson parametric correlation test (r).

RESULTS AND DISCUSSION

Protein content, amino acid composition and score of lentils

Hang et al. (2022) presented protein content profiles for the lentil diversity panel grown in multiple western Canadian environments ($n = 1290$), and values ranged from 22.1% to 34.6%. The protein content range presented by this previous study was used to select the subset of 40 samples used in the current work. Studies show similar results for lentil protein (23%–36%) (Cargo-Froom et al., 2022; Rathod & Annapure, 2016; Subedi et al., 2020), confirming this pulse as an important plant-based protein source.

The amino acid nutritional requirement is defined as the lowest level of dietary indispensable amino acid intake (in protein form) that will balance the losses of nitrogen from the body and maintain the body's protein mass. For pregnant and lactating women and children, this also includes the need for milk secretion and tissue deposition (Boye et al., 2012). A different intake level of dietary amino acids is needed to maintain body health for each life stage. Reports from the joint FAO/WHO Expert Consultation on Protein Quality Evaluation (FAO/WHO, 1991, 2007, 2013) have established the recommended scoring patterns (at various times), which were used to calculate protein quality.

The recommended amino acid scoring by FAO/WHO for pre-school children (2–5 years) (FAO/WHO, 1991), infants (birth to 6 months), young children (6 months to 3 years), and older children, adolescents, and adults (FAO/WHO, 2013), are presented in Table 1. The pre-school children (2–5 years) amino acid scoring pattern is still the required pattern to determine the PDCAAS and establish protein claims in USA regulations (FDA, 2018). Comparing values suggested for pre-school children (1991) and young children (2013), the requirements for essential amino acids varied, except for LEU. The major reductions for young children (6 months to 3 years) were for TRP (from 11.0 to 8.5 mg/g protein), aromatic amino acids (PHE + TYR, from 63.0 to 52.0 mg/g protein), and THR (from 34.0 to 31 mg/g protein), while the main increases were for VAL (from 35.0 to 43.0 mg/g protein),

TABLE 1 Recommended amino acid scoring by FAO/WHO for pre-school children (2–5 years), infants (birth to 6 months), young children (6 months to 3 years), and older children, adolescents, and adults.

	(mg/g protein)								
AA reference values from FAO/WHO	**HIS**	**ILE**	**LEU**	**LYS**	**THR**	**TRP**	**VAL**	**MET + CYS**	**PHE + TYR**
1991, pre-school children (2–5 years)	19	28	66	58	34	11	35	25	63
2013, for infant (birth to 6 months)	21	55	96	69	44	17	55	33	94
2013, for young children (6 months to 3 years)	20	32	66	57	31	8.5	43	27	52
2013, older children, adolescents, and adults	16	30	61	48	25	6.6	40	23	41

ILE (from 28.0 to 32.0 mg/g protein), and sulfur amino acids (MET+CYS, from 25.0 to 27.0 mg/g protein). When comparing the reference values for adults and infants (FAO/WHO, 2013), there is an increase in the requirement for all amino acids, as expected, due to the infants' higher amino acid intake needs to support protein accretion.

The essential and nonessential amino acid composition of lentil samples was evaluated in Hang et al. (2022) study. Table S1 presents a summarized version of the essential and non-essential amino acid composition results for the lentil diversity panel samples ($n = 1290$) obtained by NIR, including minimum and maximum values, mean, standard variation, and coefficient of variation (Hang et al., 2022). Based on the FAO/WHO (2013) age-categories scoring pattern for young children and older children, adolescents, and adults, the sulfur amino acids (MET + CYS) were the first-limiting amino acids for all samples, while for infants, 99.9% of the samples had tryptophan as the first-limiting amino acid. Tryptophan was also the first-limiting amino acid for 63.5% of the samples for FAO/-WHO (1991) pre-school children as the scoring pattern reference, and 36.5% were sulfur amino acids. This demonstrates that the impact of appropriate reference pattern selection is vital because the reference values inherently modify the overall amino acid score, the first-limiting amino acid, and the *in vitro*-protein digestibility corrected amino acid score (IV-PDCAAS) (Nosworthy & House, 2017). As such, this will have implications for establishing protein content claims within various jurisdictions.

Figure 1a–i presents the comparison of the frequency distribution of the lentil samples' amino acid scores according to the recommended amino acid scoring by FAO/WHO for pre-school children (2–5 years), infants (birth to 6 months), young children (6 months to 3 years), and older children, adolescents, and adults, for each essential amino acid. The differences are well established when comparing scoring patterns by FAO/WHO (2013) for infants and adults. However, scoring patterns for pre-school children (2–5 years) (FAO/WHO, 1991) and young children (FAO/WHO, 2013) show subtle shifts. The graphs also show that tryptophan (Figure 1f) and sulfur amino acids (Figure 1h) are the most limiting amino acids for the lentil samples in these reference groups. However, analyzing the FAO/WHO (2013) for adults, the amino acid score for tryptophan is higher than 100% (therefore, not limiting), while for sulfur amino acids (MET + CYS), they are still limiting amino acids. Attention must be paid to ILE (Figure 1b) and VAL (Figure 1g) due to the fact that their amino acid scores for adults (FAO/WHO, 2013) are lower than the AAS values of the reference for pre-school children (2–5 years) (FAO/WHO, 1991). This work demonstrates the impacts of using a specific amino acid reference pattern on the protein quality of a determined plant-based protein source.

Amino acid composition (g/100 g sample) of the subset of lentil samples ($n = 40$) from wet chemistry is stored on the KnowPulse website (https://knowpulse.usask.ca/study/AA-Scoring-Patterns-Affect-Nutritional-Claims-of-Lentils). These results are also summarized and presented in Table S2 (see Supplementary Materials). These data were used to calculate the amino acid score according to FAO/WHO different scoring patterns (Tables S3–S6). The amino acid score calculated was used to estimate the AAS-IVPDC results, further discussed in the next section.

In vitro protein digestibility and AAS-IVPDC of lentils

The 40 lentil samples were used to evaluate protein quality and digestibility. Table S7 shows the protein content, IVPD and AAS-IVPDC results for each lentil sample evaluated (see Supplementary Materials). Table 2 presents a summarized version of these results, including minimum and maximum values, mean, standard variation, and coefficient of variation. The protein content varied from 24.3% to 34.6%. The lentil samples' *in vitro* protein digestibility was 82.6 ± 1.1%. Studies showed similar IVPD results for unprocessed lentils, such as 82.5% (Barbana & Boye, 2013) and 81.8% (Sulieman et al., 2008). The Analysis of Variance (ANOVA) for the IVPD and AAS-IVPDC results are presented in Table S8 (see Supplementary Materials). The Pearson parametric correlation test (r) was used to verify how lentils' protein content affects protein digestibility and if there is an association between these two data

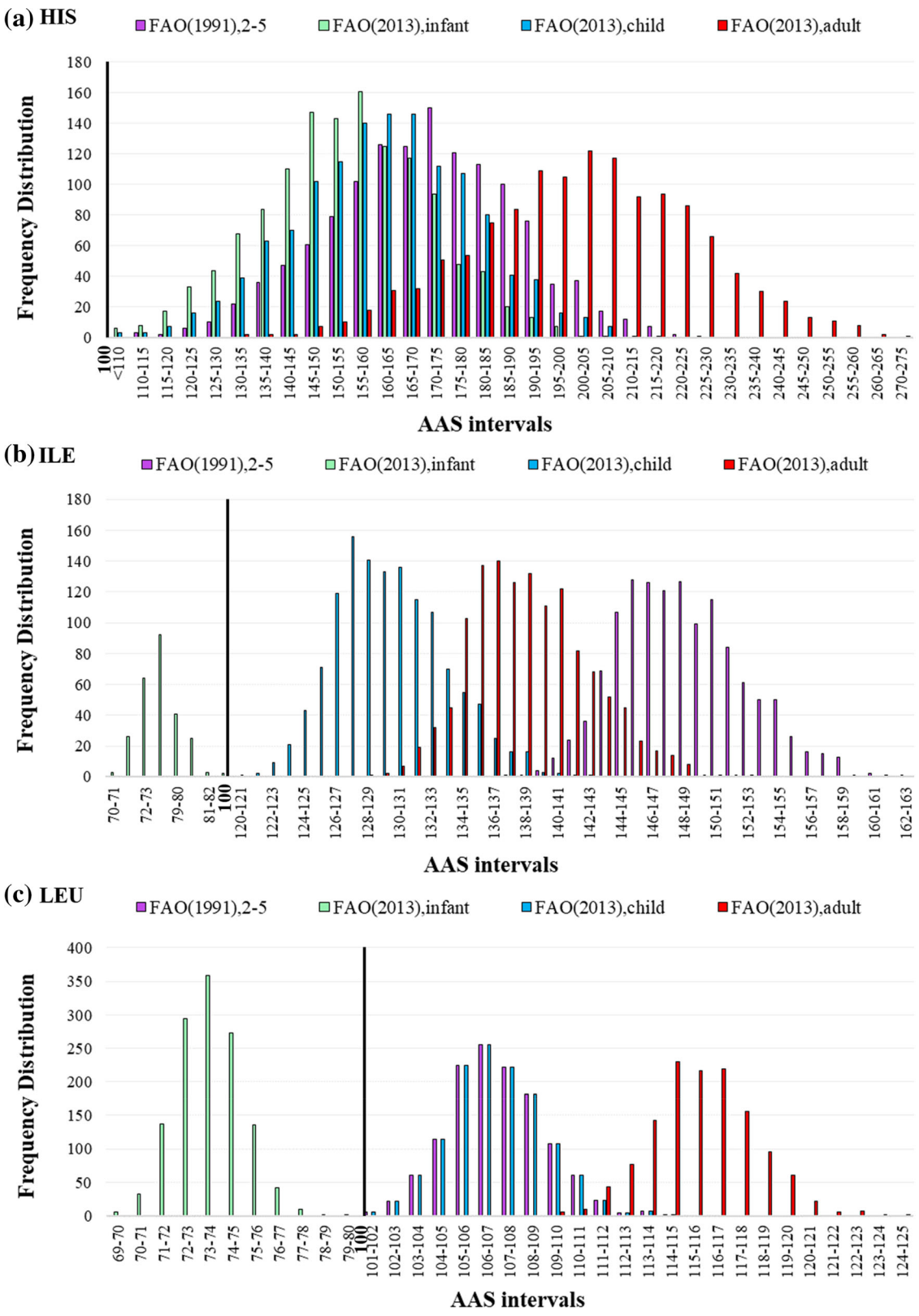

FIGURE 1 Amino acid score (AAS) frequency distribution of lentil samples ($n = 1290$) according to the recommended amino acid scoring by FAO/WHO for pre-school children (2–5 years), infants (birth to 6 months), young children (6 months to 3 years), and older children, adolescents and adults, for each essential amino acid: (a) histidine; (b) isoleucine; (c) leucine; (d) lysine; (e) threonine; (f) tryptophan; (g) valine; (h) sulfur amino acids, methionine and cysteine; and (i) aromatic amino acids, phenylalanine and tyrosine. Observation: there are gaps in the AAS intervals (abscissa) because no sample has these values.

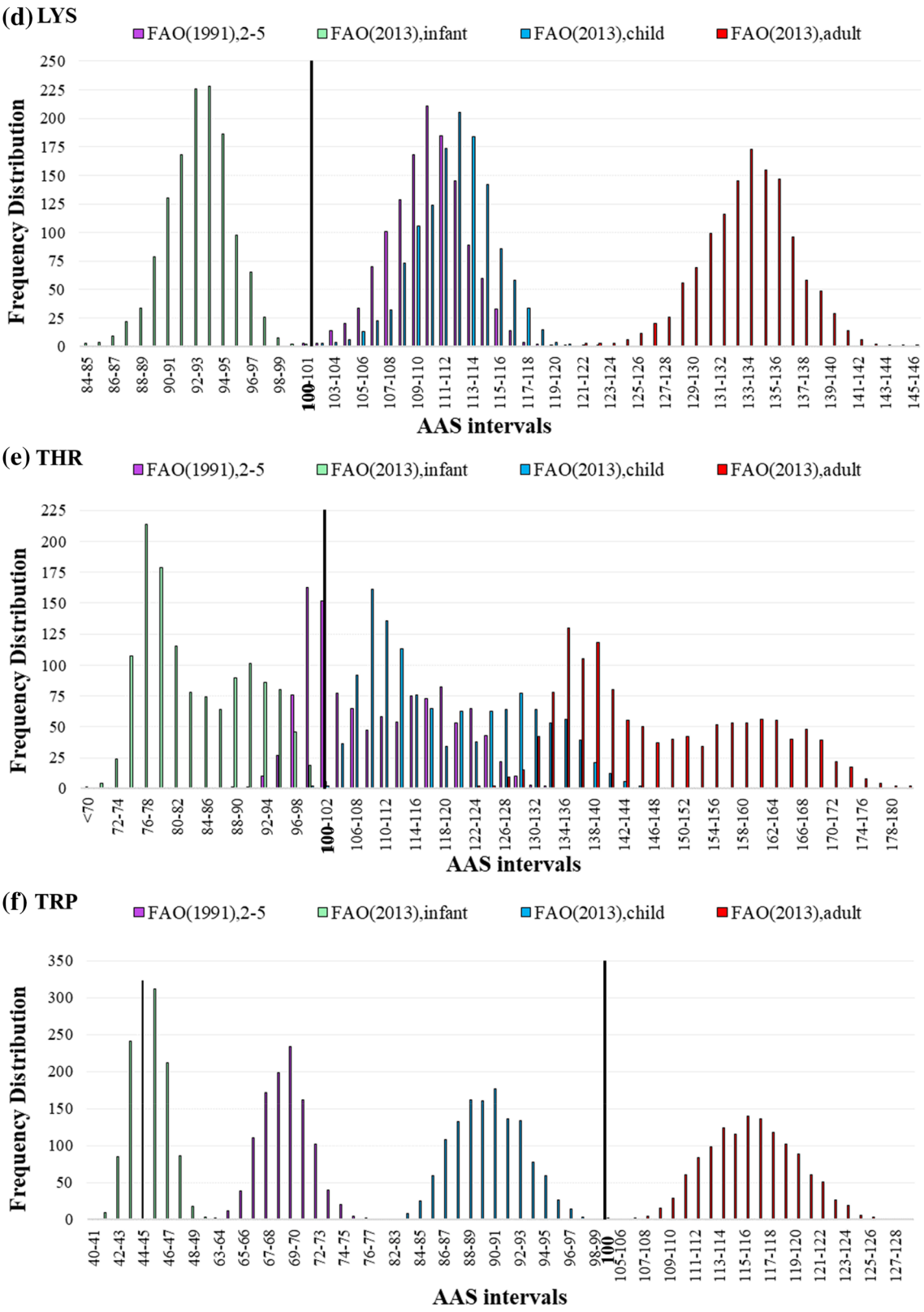

FIGURE 1 (Continued)

sets. The results presented statistical significance ($p < 0.05$), where the protein content accounted for 39% of the variance in protein digestibility ($r = 0.62$ and $R^2 = 0.39$). Figure S1 presents the correlation between *in vitro* protein digestibility (%) and protein content (%) of lentils for a subset of samples ($n = 40$). Generally, pulse

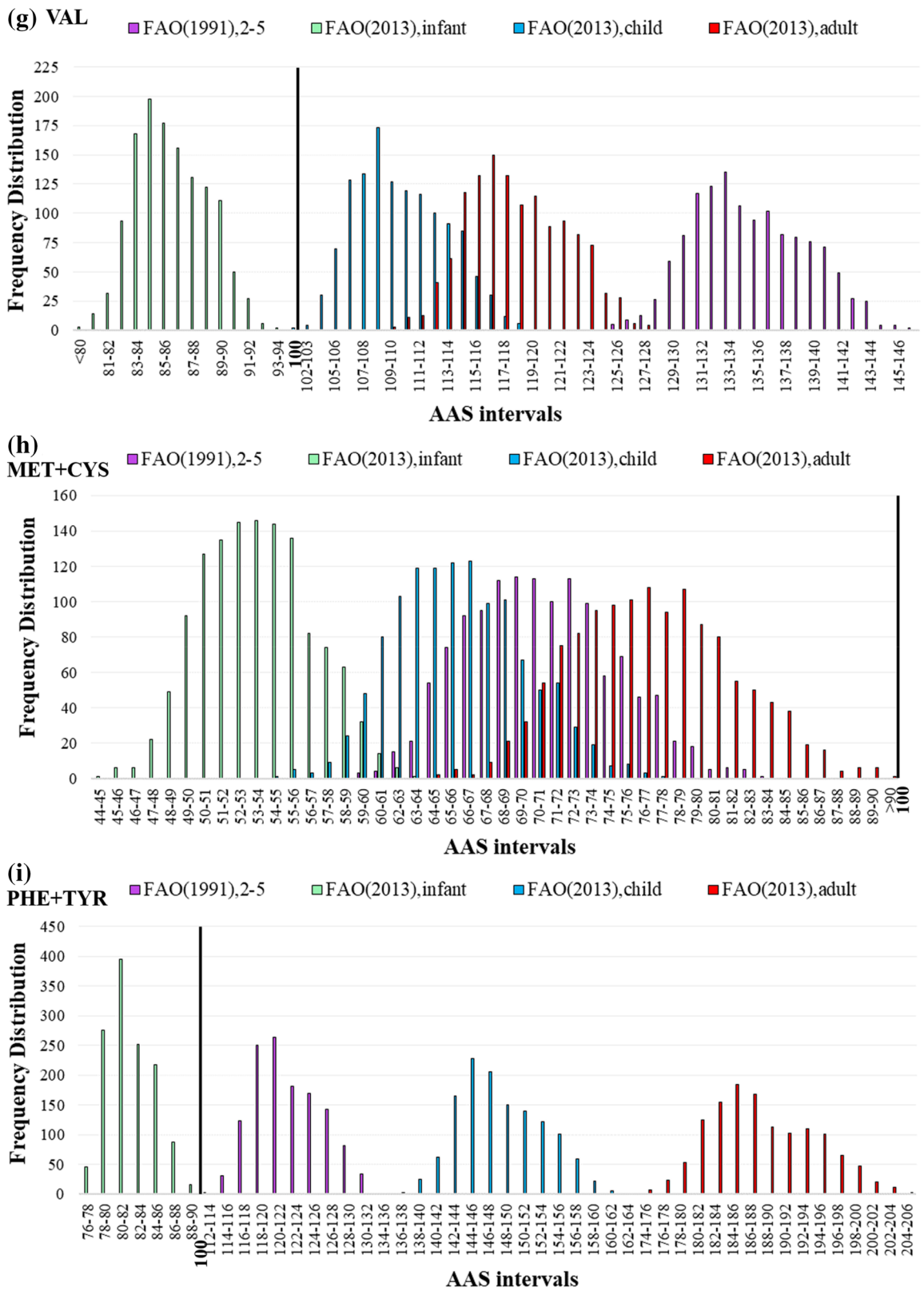

FIGURE 1 (Continued)

proteins are classified into two major fractions—globulins and albumins—where globulins are the major storage proteins, constituting 60%–80% of total protein, and the remaining protein fraction mainly consists of albumins (15%–25%) (Agarwal, 2017; Singh, 2017). The globulin fraction usually has more glutamic acid, arginine, and lysine. Albumin fractions have a higher amount of methionine and cystine. Studies have demonstrated that

TABLE 2 Protein content, IVPD and AAS-IVPDC results for lentil samples.

Lentil samples ($n = 40$)					
Protein quality evaluation	**Min**	**Max**	**Mean**	**SD**	**CV (%)**
Protein content (%)	24.3	34.6	29.7	1.8	5.9
IVPD (%)	80.2	84.9	82.6	1.1	1.4
AAS-IVPDC[a] (%)	53.1	61.0	57.5	1.8	3.2
AAS-IVPDC[b] (%)	35.5	39.8	37.5	1.0	2.7
AAS-IVPDC[c] (%)	49.2	60.5	54.2	2.8	5.1
AAS-IVPDC[d] (%)	57.7	74.0	64.0	3.4	5.3

Abbreviations: AAS-IVPDC, amino acid score corrected for *in vitro* protein digestibility; CV, coefficient of variation; IVPD, *in vitro* protein digestibility; Max, maximum value; Min, minimum value; SD, standard deviation.
[a]AAS-IVPDC in this row was calculated: IVPD × AAS [using FAO/WHO, 1991 amino acid scoring pattern for pre-school children (2–5 years)].
[b]AAS-IVPDC in this row was calculated: IVPD × AAS [using FAO/WHO, 2013 amino acid scoring pattern for infants (birth to 6 months)].
[c]AAS-IVPDC in this row was calculated: IVPD × AAS [using FAO/WHO, 2013 amino acid scoring pattern for young children (6 months to 3 years)].
[d]AAS-IVPDC in this row was calculated: IVPD × AAS [using FAO/WHO, 2013 amino acid scoring pattern for older children, adolescents, and adults].

globulins from lentils had better protein digestibility when compared to albumins due to the presence of lower cystine content (i.e., a smaller number of disulfide bonds) (Singh, 2017). Globulins' digestibility from various legume seeds is also high because they are sensitive to proteolysis (Mariotti et al., 2001). Thus, the high protein content in lentils reflects the high storage protein fraction content (e.g., globulins), which influences the amino acid composition, and, therefore, the *in vitro* protein digestibility.

The AAS-IVPDC[1] was calculated as a product of the lowest AAS and IVPD, using FAO/WHO (1991) scoring patterns for pre-school children (2–5 years), and these results are also presented in Table 2, varying from 53.1% to 61.0% for raw samples. Studies showed similar IV-PDCAAS results for other processed pulses, such as yellow pea (extruded, 62.3%) (Nosworthy, Franczyk, Medina, et al., 2017), fababean (baked, 57.5%), black bean (cooked, 62.3%), and navy bean (extruded, 55.3%) (Nosworthy et al., 2018a).

Although animal experimentation (e.g., *in vivo* methods) is required by governmental regulations for the determination of protein quality, recent studies suggest strong correlations between *in vivo* and *in vitro* measurements of protein digestibility and protein quality (e.g., PDCAAS). Nosworthy, Franczyk, Medina, et al. (2017) evaluated the impact of processing on the protein quality of pinto bean and buckwheat flour, and *in vivo* PDCAAS was compared to the *in vitro* approach. A good correlation was found ($R^2 = 0.9280$). Tavano et al. (2016) evaluated the correlation between *in vitro* and *in vivo* PDCAAS for chickpea fractions and showed that *in vitro* protein digestibility can be applied to calculate *in vitro* PDCAAS (i.e., a similar approach to AAS-IVPDC[1] in this study) with strong correlations compared to *in vivo* tests ($R^2 = 0.9442$). For red and green lentils, Nosworthy et al. (2018a) demonstrated strong correlation between *in vitro* and *in vivo* PDCAAS ($R^2 = 0.9971$). Nosworthy et al. (2018b) investigated *in vivo* and *in vitro* protein digestibility and quality using beans and found *in vivo* PDCAAS, and *in vitro* PDCAAS to be significantly correlated ($R^2 = 0.7497$) (Nosworthy et al., 2018a). Furthermore, a study was performed comparing *in vivo* and *in vitro* measurements of digestibility, and protein quality for protein concentrates and isolates from faba bean, lentil, and pea, and a strong correlation ($R^2 = 0.9898$) was found between *in vivo* PDCAAS and *in vitro* PDCAAS (Nosworthy & House, 2017). Although both *in vitro* and *in vivo* PDCAAS use a common amino acid score, the strong correlation between these values suggests that *in vitro* PDCAAS could be used as a surrogate for *in vivo* evaluation of pulse protein ingredients for determining protein quality (Nosworthy et al., 2018b; Nosworthy, Franczyk, Zimoch-Korzycka, et al., 2017). The advantages of the *in vitro* approach were convenience, simplicity, lower cost, increased speed, and importantly, reduced animal usage. The use of *in vitro* methods for digestibility analysis deserves further study and discussion, with a special focus on seeking method standardization (Tavano et al., 2016).

Lentil, with the lowest AAS and IVPD using scoring patterns for infants (birth to 6 months), young children (6 months to 3 years), and older children, adolescents, and adults (FAO/WHO, 2013), present different scores (AAS-IVPDC[2], AAS-IVPDC[3], and AAS-IVPDC[4], respectively), presented in Table 2. The results were 37.5 ± 1.0%, 54.2 ± 2.8%, and 64.0 ± 3.4% for the three scoring patterns, respectively. When comparing the same lentil sample, the results for AAS-IVPDC[3] (younger children) were lower than those of AAS-IVPDC[1] (Table S7) in a similar age category, emphasizing that the choice of the FAO/WHO amino acid scoring patterns can impact the protein quality of lentils or other plant-based protein sources, especially when moving to FAO/WHO (2013) scoring patterns. As the same protein digestibility and amino acid profile were used for AAS-IVPDC calculations, the difference between these values occurs specifically due to the change in the amino acid

TABLE 3 Impact of AAS-IVPDC and using the interpretation of PDCAAS and DIAAS systems for determining protein content claims for lentils with the United States dietary standards.

Samples	AAS-IVPDC[a]	Corrected protein content in RACC[b] (g)	Permitted protein claim[c]	AAS-IVPDC[d]	Corrected protein content in RACC[e] (g)	Permitted protein claim[f]	AAS-IVPDC[g]	Corrected protein content in RACC[e] (g)	Permitted protein claim[f]	AAS-IVPDC[h]	Corrected protein content in RACC[e] (g)	Permitted protein claim[f]
1	60.2	5.1	Good source	39.0	3.3	No claim	56.7	4.8	No claim	66.6	5.7	No claim
2	57.7	5.6	Good source	37.7	3.7	No claim	53.5	5.2	No claim	62.8	6.1	No claim
3	57.1	5.6	Good source	37.0	3.6	No claim	53.4	5.2	No claim	62.7	6.1	No claim
4	58.9	5.6	Good source	38.1	3.6	No claim	59.0	5.6	No claim	69.2	6.6	No claim
5	58.1	5.6	Good source	38.0	3.7	No claim	53.8	5.2	No claim	63.1	6.1	No claim
6	56.0	5.5	Good source	36.3	3.6	No claim	52.5	5.2	No claim	61.6	6.1	No claim
7	56.7	5.6	Good source	36.7	3.6	No claim	53.5	5.2	No claim	62.9	6.2	No claim
8	57.5	5.7	Good source	37.2	3.7	No claim	54.5	5.4	No claim	64.0	6.3	No claim
9	57.5	5.7	Good source	37.2	3.7	No claim	53.2	5.3	No claim	62.5	6.2	No claim
10	55.1	5.9	Good source	36.7	3.9	No claim	51.1	5.4	No claim	59.9	6.4	No claim
11	57.9	5.8	Good source	37.4	3.7	No claim	55.6	5.6	No claim	65.3	6.5	No claim
12	59.6	6.0	Good source	38.6	3.9	No claim	56.1	5.6	No claim	72.4	7.2	No claim
13	58.1	5.8	Good source	37.6	3.8	No claim	56.5	5.7	No claim	66.3	6.6	No claim
14	54.2	5.5	Good source	36.1	3.6	No claim	50.1	5.0	No claim	58.9	5.9	No claim
15	56.6	5.7	Good source	36.6	3.7	No claim	50.0	5.0	No claim	63.7	6.4	No claim
16	59.2	6.0	Good source	38.9	4.0	No claim	54.8	5.6	No claim	64.4	6.6	No claim
17	57.6	5.9	Good source	37.3	3.8	No claim	54.0	5.5	No claim	63.4	6.5	No claim
18	57.7	5.9	Good source	37.4	3.8	No claim	54.4	5.6	No claim	63.9	6.5	No claim
19	59.2	6.0	Good source	38.3	3.9	No claim	57.6	5.9	No claim	67.7	6.9	No claim
20	57.2	5.9	Good source	37.0	3.8	No claim	55.2	5.7	No claim	64.8	6.7	No claim
21	58.6	6.1	Good source	37.9	4.0	No claim	58.4	6.1	No claim	68.5	7.2	No claim
22	54.9	5.7	Good source	37.0	3.8	No claim	50.8	5.3	No claim	59.7	6.2	No claim
23	56.5	5.9	Good source	36.6	3.8	No claim	52.9	5.5	No claim	62.1	6.5	No claim
24	58.7	6.2	Good source	38.0	4.0	No claim	60.3	6.4	No claim	70.8	7.5	No claim
25	58.5	6.1	Good source	38.1	4.0	No claim	54.1	5.7	No claim	63.5	6.6	No claim
26	57.1	6.1	Good source	37.0	3.9	No claim	53.6	5.7	No claim	62.9	6.7	No claim
27	59.6	6.3	Good source	38.5	4.1	No claim	57.6	6.1	No claim	67.6	7.2	No claim
28	59.2	6.3	Good source	38.3	4.1	No claim	55.0	5.8	No claim	64.6	6.8	No claim
29	59.4	6.3	Good source	38.5	4.1	No claim	56.3	6.0	No claim	66.1	7.0	No claim
30	55.8	5.9	Good source	36.4	3.9	No claim	51.6	5.5	No claim	60.6	6.4	No claim
31	55.9	6.1	Good source	36.1	3.9	No claim	52.3	5.7	No claim	61.4	6.7	No claim
32	60.2	6.6	Good source	39.4	4.3	No claim	55.8	6.1	No claim	65.5	7.2	No claim

(Continues)

TABLE 3 (Continued)

Samples	AAS-IVPDC[a]	Corrected protein content in RACC[b] (g)	Permitted protein claim[c]	AAS-IVPDC[d]	Corrected protein content in RACC[e] (g)	Permitted protein claim[f]	AAS-IVPDC[g]	Corrected protein content in RACC[e] (g)	Permitted protein claim[f]	AAS-IVPDC[h]	Corrected protein content in RACC[e] (g)	Permitted protein claim[f]
33	53.6	5.9	Good source	35.8	3.9	No claim	49.6	5.4	No claim	58.2	6.4	No claim
34	58.6	6.5	Good source	39.1	4.4	No claim	54.2	6.0	No claim	63.7	7.1	No claim
35	54.3	6.1	Good source	36.6	4.1	No claim	50.3	5.6	No claim	59.0	6.6	No claim
36	53.8	6.1	Good source	36.4	4.1	No claim	49.8	5.6	No claim	58.4	6.6	No claim
37	57.4	6.5	Good source	37.2	4.2	No claim	56.9	6.4	No claim	66.8	7.5	No claim
38	57.1	6.1	Good source	39.2	4.2	No claim	50.6	5.4	No claim	62.0	6.7	No claim
39	59.3	6.6	Good source	38.4	4.2	No claim	58.3	6.4	No claim	68.4	7.6	No claim
40	57.5	7.0	Good source	37.3	4.5	No claim	53.2	6.5	No claim	62.5	7.6	No claim

Abbreviations: AAS-IVPDC, amino acid score corrected for *in vitro* protein digestibility; RACC, reference amount customarily consumed.

[a]AAS-IVPDC (%) in this column was calculated: IVPD × AAS [using FAO/WHO (1991) amino acid scoring pattern for pre-school children (2–5 years)].

[b]Corrected protein content = crude protein content in RACC × IV-PDCAAS, while RACC from FDA: 21 CFR 101.12 (2015) for legumes/pulses: 35 g (dry).

[c]Claims of 'Good source': 5.0–9.9 g per RACC; 'Excellent source': ≥ 10 g per RACC, which is a similar interpretation to PDCAAS system (FDA, 21 CFR 101.12, 2015).

[d]AAS-IVPDC (%) in this column was calculated: IVPD × AAS [using FAO/WHO (2013) amino acid scoring pattern for infants (birth to 6 months)].

[e]Corrected protein content = crude protein content in RACC × IV-DIAAS, while RACC from FDA: 21 CFR 101.12 (2015) for legumes/pulses: 35 g (dry).

[f]Claims of 'Good source': 5.0–9.9 g per RACC and AAS-IVPDC > 75%; 'Excellent source': ≥ 10 g per RACC and AAS-IVPDC ≥ 100%, which is a similar interpretation to DIAAS system (FAO/WHO, 2013).

[g]AAS-IVPDC (%) in this column was calculated: IVPD × AAS [using FAO/WHO (2013) amino acid scoring pattern for young children (6 months to 3 years)].

[h]AAS-IVPDC (%) in this column was calculated: IVPD × AAS [using FAO/WHO (2013) amino acid scoring pattern for older children, adolescents, and adults].

reference pattern, particularly for tryptophan and sulfur amino acids, which are usually the most limiting amino acids for lentil (and other legumes). The AAS-IVPDC[1] requirement for MET+CYS is 25 mg/g protein, while the AAS-IVPDC[3] requirement for young children (similar age category) is 27 mg/g protein. Regarding TRP, the IV-PDCAAS requirement is 11 mg/g protein and 8.5 mg/g protein for AAS-IVPDC[3] for young children (Table 1) (FAO/WHO, 1991, 2013). These changes in guidelines directly impact the amino acid value for AAS-IVPDC.

Table 3 presents the impact of using the AAS-IVPDC results and a similar interpretation of PDCAAS and DIAAS systems for determining protein content claims with the United States dietary standards for each lentil sample ($n = 40$). In the United States, PDCAAS and the "Corrected Protein Content in RACC" (FDA, 2015) are used to make protein claims qualifications, such as "good source" if the corrected content is 5.0–9.9 g per RACC and "excellent source" if it is ≥ 10 g per RACC. All lentil samples had the permitted protein claim of "good source" when using the AAS-IVPDC[1] method. Considering the recommendations in the FAO/WHO (2013) report, all samples were disadvantaged when using AAS-IVPDC[2], AAS-IVPDC[3], and AAS-IVPDC[4] results due to failing to meet the claim threshold of 75%, currently used in the DIAAS system (Table 3) despite many samples matching values for corrected protein content in the RACC. This cut-off value for establishing protein content claims must continue to be evaluated in the context of national food-based dietary guidelines (FBDGs). Nutrition and food knowledge is essential in increasing consumer awareness about high-protein foods and comprehending how to increase them in their diet within a gradually complex food environment (Fernandez et al., 2020). Thus, the FBDGs exist for guidance about food, food groups, and dietary patterns that optimize nutrient intake, promote health, and prevent chronic diseases for healthy individuals (Health Canada, 2019b). Canada's Food Guide (2019) emphasizes consuming diets that include proteins from a variety of sources—largely plant-based (legumes, nuts, and seeds) and animal proteins (fish, meat, and dairy foods)—to provide a variety of nutrients in sufficient amounts to the diet, with limited amounts of refined grains, processed foods, and added sugars (Fernandez et al., 2020). Nevertheless, if one adopts a claim threshold of 75% as the suggested regulatory benchmark for stipulating protein content claims, several proteins from seeds, nuts, or pulse sources would disappear from the ideal plate represented in Canada's Food Guide (2019), and it would be mainly composed of animal proteins—inconsistent with current food guidelines.

Furthermore, studies evaluating the impact of the threshold of 75% on protein content claims of different plant-based sources reinforce that it is not only lentils will be impacted. Many other plant-based foods will also come up short. Marinangeli and House (2017) evaluated different pulses (e.g., navy beans, yellow peas, split red lentils) and soy products (e.g., tofu) that have 'good source of protein' claims using the interpretation of PDCAAS method. However, none of these products had protein claims due to the DIAAS threshold of 75%. Thus, several plant-based foods can be disadvantaged by the recommended policy changes, and with such an impact on protein content, it is important to have an accurate and universal protein quality evaluation for food labeling. Additionally, no investigation has yet shown that not meeting the threshold of 75% would lead to inadequate protein intake in older children and adults. Therefore, before implementing FAO/WHO (2013) recommendations, a critical appraisal must be conducted on the potential impact on national dietary guidelines. An updated framework that distinguishes different protein sources and better interprets protein quality (particularly for plant and mixed-protein sources) is needed to avoid misleading health claims and to encourage transparency when marketing new food trends.

CONCLUDING REMARKS

In a growing global population, attention must be paid to food security and economically feasible protein source development. Plant protein characterization becomes a necessity as more individuals seek alternative protein sources. High-quality protein demand requires reliable protein quality determination. Careful selection of an appropriate amino acid scoring pattern and protein quality assessment for plants and plant-based food labeling is vital. Determining lentils' protein and amino acid composition can benefit lentil breeding programs leading to improved protein quality, helping food industries create value-added products with new food formulations, and making correct nutrient content claims. The high protein content of lentils and their utility in new food formulations places this pulse crop as a promising alternative high-quality protein supply for future generations. However, more work is necessary to understand the most appropriate amino acid scoring pattern for humans of all ages. As demonstrated here, choosing a specific age-category amino acid scoring pattern can directly impact perceived protein quality. Currently, stakeholders using different scoring patterns can negatively influence marketplace purchases with their protein content claims. Therefore, an amino acid scoring pattern must be based on scientific evidence describing the requirements of an individual, and it should not be able to be manipulated to achieve different outcomes.

Protein quality determination can have vast implications for jurisdictional regulatory frameworks,

consumer-facing nutrition communication, and public health and well-being. Harmonizing protein quality evaluation processes and interpretation between Canada and the United States is a key issue remaining to be solved, given the strong integration of food supply chains. Furthermore, food policies and protein content claims must be thoroughly evaluated and revised. Adopting arbitrary criteria set for making protein content claims (threshold of 75%) could impact the ability of a given food, such as legumes and other plant-based protein foods, to qualify for protein content claims and thus affect consumers' food choices. Thus, it is crucial to consider the potential impacts of this shift on regulatory decisions before its adoption. In this study, even using a wide selection of lentil samples (representing a large variety of lentil genotypes), lentils still do not qualify for protein content claims if using the interpretation of the DIAAS cut-off of 75%. Moreover, as depicted in the new Canada's Food Guide (2019), an ideal plate for healthy eating each day should contain plenty of vegetables and fruits, whole-grain foods, and diverse protein sources. However, if one considers the FAO/WHO (2013) recommended regulatory benchmarks for establishing 'Sources of Protein', this Canada's Food Guide ideal plate would be mainly composed of animal proteins and soybean (e.g., tofu), inconsistent with current food guideline recommendations. For this reason, a critical appraisal must be conducted to assess the impact this criterion used for making protein content claims. The suggestion here is that FAO/WHO (2013) recommendations should be revised based on the intended use of protein food in the population (e.g., non-medical foods or consumed by children or the elderly) and use a similar approach to the U.S. system, based on the percent daily value (DV) for protein per reference amount customarily consumed (RACC) and the corrected protein content. Using inconsistent methods can directly influence protein content claims for pulses, challenging dietary guidance globally, especially in North America, where protein quality defines the protein content claims of foods.

The requirement to use animal models for the DIAAS measurement, particularly the ileal-cannulated pig, presents major challenges to understanding the environmental, genetic and processing factors that can influence the protein quality of crops, such as lentil. As such, alternative approaches are being developed for *in vitro* protein and amino acid digestibility assessments. However, there are limitations to using *in vitro* protein digestibility as a proxy for ileal amino acid digestibility. The *in vitro* methods are still in their infancy, particularly for plant-based protein sources. Sousa et al. (2023) analyzed the *in vitro* protein digestibility of plant-based burgers (e.g., soybean and pea-fababean) according to the INFOGEST protocol (Brodkorb et al., 2019) and calculated the digestible indispensable amino acid score (DIAAS) based on *in vitro* digestibility. The grilled soybean protein-based burger reached 94.0% for *in vitro* DIAAS values, and the sulfur amino acids were the first-limiting amino acids in the plant-based burger.

Furthermore, Shaheen et al. (2016) reported DIAAS values for lentils as 46% (using FAO/WHO (2013) amino acid scoring pattern for young children) and 54% (using FAO/WHO (2013) amino acid scoring pattern for older children, adolescents, and adults). In the current study, AAS-IVPDC^{3} values were 49.2%–60.5% (young children) and for AAS-IVPDC^{4} were 57.7%–74.0% (adults), similar to the *in vivo* values reported in the literature. The suggestion is using fixed estimates of ileal digestibility or using *in vitro* methods to determine protein digestibility rather than *in vivo*. In 2022, the USA Congress passed the FDA Modernization Act 2.0 (Bill S.2952), which removes the requirement for pharmaceutical companies to test drugs on animals before human trials, thus creating more momentum to find suitable *in vitro* methods to determine digestibility coefficients for both PDCAAS and DIAAS. This is an additional impetus for a thorough and pragmatic assessment before any widespread adoption of DIAAS as a benchmark for protein quality within regulatory and policy frameworks.

A question remains whether implementing DIAAS for protein source claims would make foods that already qualify (based on the PDCAAS in US or PER in Canada) ineligible. This consequence must be considered when proposing any nutrient content claim directing consumers toward foods that contribute to daily nutrient requirements. As shown in Table 3, if one focuses on the absolute amount of corrected protein content in RACC, using both AAS-IVPDC^{1}, AAS-IVPDC^{3}, and AAS-IVPDC^{4}, it would yield similar results and protein claims, except for AAS-IVPDC^{2} which was calculated with the amino acid scoring pattern for infants (birth to 6 months) (FAO/WHO, 2013). Therefore, at a minimum, the impact of the seemingly arbitrary cut-off of 75% for making a protein content claim needs to be re-evaluated. New scientific data will be required to support policy and inform expert recommendations globally to keep guidelines and legislation relevant.

AUTHOR CONTRIBUTIONS

Amanda G. A. Sá: Conceptualization, Formal analysis, Methodology, Investigation, Writing—original draft. **Jiayi Hang**: Formal analysis, Methodology, Investigation. **Laura Jardine**: Writing—review & editing. **Kirstin E. Bett**: Project administration, Source of lentil samples, Writing—review & editing, Funding acquisition. **James D. House**: Supervision, Conceptualization, Project administration, Writing—review & editing, Funding acquisition. All authors contributed to and approved the final draft of the manuscript.

ACKNOWLEDGMENTS

The authors thank PerkinElmer Health Sciences Canada Inc. (Winnipeg, Canada) for the utilization of

the near-infrared spectroscopy (NIR) equipment for amino acid composition determination.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Amanda G. A. Sá https://orcid.org/0000-0003-4654-7426
James D. House https://orcid.org/0000-0003-1389-5491

REFERENCES

Agarwal A. Proteins in pulses. J Nutr Disord Ther. 2017;7(1):2161. https://doi.org/10.4172/2161-0509.1000e129

AOAC International. In: Latimer GW, editor. Official methods of analysis of AOAC international. 19th ed. Rockville, Maryland: AOAC International; 2012.

Barbana C, Boye JI. In vitro protein digestibility and physico-chemical properties of flours and protein concentrates from two varieties of lentil (*Lens culinaris*). Food Funct. 2013;4(2):310–21. https://doi.org/10.1039/c2fo30204g

Boye J, Wijesinha-Bettoni R, Burlingame B. Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. Br J Nutr. 2012;108: S183–211. https://doi.org/10.1017/S0007114512002309

Brodkorb A, Egger L, Alminger M, Alvito P, Assunção R, Ballance S, et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc. 2019;14(4):991–1014. https://doi.org/10.1038/s41596-018-0119-1

Canadian Food Inspection Agency. Reasonable daily intake for various foods (Schedule K). 2021a Available from: https://inspection.canada.ca/food-labels/labelling/industry/nutrition-labelling/nutrition-facts-table/eng/1389198568400/1389198597278?chap=6. Accessed 10 Aug 2022. Government of Canada

Canadian Food Inspection Agency. Specific nutrient content claim requirements. 2021b Available from: https://inspection.canada.ca/food-labels/labelling/industry/nutrient-content/specific-claim-requirements/eng/1627085614476/1627085788924?chap=9. Accessed 8 Oct 2022. Government of Canada

Cargo-Froom CL, Newkirk RW, Marinangeli CPF, Shoveller AK, Ai Y, Kiarie EG, et al. The effects of grinding and pelleting on nutrient composition of Canadian pulses. Can J Anim Sci. 2022;102(3): 1–38. https://doi.org/10.1139/cjas-2021-0111

FAO/WHO. Protein quality evaluation: Report of the joint FAO/WHO expert consultation. 1991 In *FAO food and nutrition paper* 51. https://www.fao.org/.

FAO/WHO. Protein and amino acid requirements in human nutrition. 2007 In *World Health Organization technical report series 935*. https://www.fao.org/

FAO/WHO. Dietary protein quality evaluation in human nutrition: Report of an FAO expert consultation. 2013 In *FAO food and nutrition paper 92*. https://www.fao.org/

Fernandez MA, Bertolo RF, Duncan AM, Phillips SM, Elango R, Ma DWL, et al. Translating protein foods from the new Canada's food guide to consumers: knowledge gaps and recommendations. Appl Physiol Nutr Metab. 2020;45(12):1311–23. https://doi.org/10.1139/apnm-2020-0192

Hang J, Shi D, Neufeld J, Bett KE, House JD. Prediction of protein and amino acid contents in whole and ground lentils using near-infrared reflectance spectroscopy. LWT—Food Sci Technol. 2022;165:113669. https://doi.org/10.1016/j.lwt.2022.113669

Health Canada. Determination of protein rating—method FO-1 (pp. 1–6). 1981 https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/measuring-protein-quality-foods.html

Health Canada. Plant-based protein market: global and Canadian market analysis. 2019a Available from: https://nrc.canada.ca/en/research-development/research-collaboration/programs/plant-based-protein-market-global-canadian-market-analysis. Accessed 10 Aug 2022

Health Canada. Revision process for Canada's food guide. 2019b Available from: https://www.canada.ca/en/health-canada/services/canada-food-guide/about/revision-process. Accessed 15 Aug 2022

Hsu HW, Vavak DL, Satterlee LD, Mmiller GA. A multienzime technique for estimating protein digestibility. J Food Sci. 1977;42: 1269–73. https://doi.org/10.1111/j.1365-2621.1977.tb14476.x

ISO. ISO 13904:2005 Animal feeding stuffs—determination of tryptophan content. 2005 Available from: https://www.iso.org/standard/37259. Accessed 9 Jan 2023

Khazaei H, Subedi M, Nickerson M, Martínez-Villaluenga C, Frias J, Vandenberg A. Seed protein of lentils: current status, progress, and food applications. Foods. 2019;8(9):391. https://doi.org/10.3390/foods8090391

Kumar J, Singh J, Kanaujia R, Gupta S. Protein content in wild and cultivated taxa of lentil (*Lens culinaris* ssp. culinaris Medikus). Indian J Genet Plant Breed. 2016;76:631–4. https://doi.org/10.5958/0975-6906.2016.00078.X

Langley G, Evans T, Holgate ST, Jones A. Replacing animal experiments: choices, chances and challenges. Bioessays. 2007; 29(9):918–26. https://doi.org/10.1002/bies.20628

Marinangeli CPF, House JD. Potential impact of the digestible indispensable amino acid score as a measure of protein quality on dietary regulations and health. Nutr Rev. 2017;75(8):658–67. https://doi.org/10.1093/nutrit/nux025

Mariotti F, Pueyo ME, Tomé D, Bérot S, Benamouzig R, Mahé S. The influence of the albumin fraction on the bioavailability and postprandial utilization of pea protein given selectively to humans. J Nutr. 2001;131(6):1706–13. https://doi.org/10.1093/jn/131.6.1706

Mariotti F, Tomé D, Mirand PP. Converting nitrogen into protein—beyond 6.25 and Jones' factors. Crit Rev Food Sci Nutr. 2008; 48(2):177–84. https://doi.org/10.1080/10408390701279749

Nosworthy MG, Franczyk A, Zimoch-Korzycka A, Appah P, Utioh A, Neufled J, et al. Impact of processing on the protein quality of pinto bean (*Phaseolus vulgaris*) and buckwheat (*Fagopyrum esculentum* Moench) flours and blends, as determined by in vitro and in vivo methodologies. J Agric Food Chem. 2017;65: 3919–25. https://doi.org/10.1021/acs.jafc.7b00697

Nosworthy MG, Franczyk AJ, Medina G, Neufeld J, Appah P, Utioh A, et al. Effect of processing on the in vitro and in vivo protein quality of yellow and green split peas (*Pisum sativum*). J Agric Food Chem. 2017;65:7790–6. https://doi.org/10.1021/acs.jafc.7b03597

Nosworthy MG, House JD. Factors influencing the quality of dietary proteins: implications for pulses. Cereal Chem. 2017;94(1): 49–57. https://doi.org/10.1094/CCHEM-04-16-0104-FI

Nosworthy MG, Medina G, Franczyk AJ, Neufeld J, Appah P, Utioh A, et al. Effect of processing on the in vitro and in vivo protein quality of beans (Phaseolus vulgaris and Vicia faba). Nutrients. 2018a;10(671):1–13. https://doi.org/10.3390/nu10060671

Nosworthy MG, Medina G, Franczyk AJ, Neufeld J, Appah P, Utioh A, et al. Effect of processing on the in vitro and in vivo protein quality of red and green lentils (*Lens culinaris*). Food Chem. 2018b; 240:588–93. https://doi.org/10.1016/j.foodchem.2017.07.129

Rathod RP, Annapure US. Effect of extrusion process on antinutritional factors and protein and starch digestibility of lentil splits. LWT—Food Sci Technol. 2016;66:114–23. https://doi.org/10.1016/j.lwt.2015.10.028

Sá AGA, Moreno YMF, Carciofi BAM. Food processing for the improvement of plant proteins digestibility. Crit Rev Food Sci Nutr. 2019;60(20):3367–86. https://doi.org/10.1080/10408398.2019.1688249

Shaheen N, Islam S, Munmun S, Mohiduzzaman M, Longvah T. Amino acid profiles and digestible indispensable amino acid

scores of proteins from the prioritized key foods in Bangladesh. Food Chem. 2016;213:83–9. https://doi.org/10.1016/j.foodchem.2016.06.057

Singh N. Pulses: an overview. J Food Sci Technol. 2017;54(4):853–7. https://doi.org/10.1007/s13197-017-2537-4

Sousa R, Portmann R, Recio I, Dubois S, Egger L. Comparison of in vitro digestibility and DIAAR between vegan and meat burgers before and after grilling. Food Res Int. 2023;166-(December 2022):112569. https://doi.org/10.1016/j.foodres.2023.112569

Subedi M, Khazaei H, Arganosa G, Etukudo E, Vandenberg A. Genetic stability and G×E interaction analysis for seed protein content and protein yield of lentil (*Lens culinaris* Medik.). Crop Sci. 2020;61(1):342–56. https://doi.org/10.1002/csc2.20282

Sulieman MA, Hassan AB, Osman GA, El Tyeb MM, El Khalil EA, El Tinay AH, et al. Changes in total protein digestibility, fractions content and structure during cooking of lentil cultivars. Pak J Nutr. 2008;7(6):801–5. https://doi.org/10.3923/pjn.2008.801.805

Tavano OL, Neves VA, Júnior SIS. In vitro versus in vivo protein digestibility techniques for calculating PDCAAS (protein digestibility-corrected amino acid score) applied to chickpea fractions. Food Res Int. 2016;1–8:756–63. https://doi.org/10.1016/j.foodres.2016.10.005

Tinus T, Damour M, Van Riel V, Sopade PA. Particle size-starch-protein digestibility relationships in cowpea (*Vigna unguiculata*). J Food Eng. 2012;113(2):254–64. https://doi.org/10.1016/j.jfoodeng.2012.05.041

U.S. Food and Drug Administration and Department of Health and Human Services. Reference amounts customarily consumed per eating occasion. 21 CFR, Part 101, Subpart A, Section 101.12. Eletronic Code of Federal Regulations. 2015 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.12

U.S. Food and Drug Administration and Department of Health and Human Services. Nutrition labeling of food. 21 CFR, Part 101, Subpart A, Section 101.9. Eletronic Code of Federal Regulations; Government Printing Office. 2018 https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-101/subpart-A/section-101.9

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sá AGA, Hang J, Jardine L, Bett KE, House JD. How different amino acid scoring patterns recommended by FAO/WHO can affect the nutritional quality and protein claims of lentils. Sust Food Prot. 2023. https://doi.org/10.1002/sfp2.1008