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Research and Regulatory Gaps for the Substantiation of Protein Content Claims on Foods

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Abstract:

Protein claims provide guidance to consumers seeking protein-rich foods. Protein claim regulations differ globally, and both Canada and the U.S. require protein quality assessments, however methods differ. A tripartite workshop identified the need to: 1) harmonize; 2) update existing amino acid composition and digestibility databases; 3) develop non-animal bioassays; and 4) evaluate the impact of protein claims on human health. The Protein-Digestibility Corrected Amino Acid Score method is recommended for current regulatory use in Canada.

Key words: protein quality; protein efficiency ratio; protein digestibility-corrected amino acid score; digestible indispensable amino acid score; nutrient content claims

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Introduction

In 2017 Health Canada released guiding principles and recommendations for healthy eating as part of the revisions to Canada's Food Guide (Health Canada, 2017). Health Canada recommended "regular intake of vegetables, fruit, whole grains and protein-rich foods, especially plant-based sources of protein". A focus on protein-rich foods is in line with recent North American trends, including a 2017 survey showing protein as a top 10 trend in food, nutrition and health interests (New Nutrition Business, 2017). Additionally, 64% of consumers actively sought food products with protein (International Food Information Council, 2016). Key drivers of this increased focus on protein-rich foods include a greater awareness of the role of protein in: a) counteracting age-related sarcopenia; b) enhancing recovery in athletes; c) contributing to satiety; and d) supporting a healthy immune system (Paddon-Jones and Leidy, 2014; Phillips et al 2016).

To fully utilize both existing and novel protein sources in the development and positioning of protein-rich foods, effective messages are required for consumer communication. In both Canada and the United States, terms such as "source of protein", "protein-rich" and "rich in protein" reflect protein content claims that require evidence to substantiate the quality of the protein. Protein quality is the ability of a protein to meet human requirements, and is impacted by the amino acid (AA) composition and digestibility of the protein. There are several methods used to estimate protein quality. A current barrier for companies seeking to position protein-containing foods within Canada and the United States is that regulatory methods for measuring protein quality differ. These differences represent barriers to innovation as reviewed in the following.

Reviewing Jurisdictional Differences in Measuring Protein Quality: The Process

To critically compare and contrast jurisdictional approaches to measuring protein quality a survey was circulated to academics, government officials, non-government organizations and food

industry stakeholders with expertise in protein quality, via the Canadian Nutrition Society and the University of Toronto Program in Food Safety, Nutrition and Regulatory Affairs (PFSNRA). Results from the expert survey¹ informed the development of a workshop (November 2016, Toronto, ON)² attended by invited representative stakeholders.

Through the tri-partite workshop, stakeholders: a) developed a research agenda designed to support the formation of evidence-based nutrition policy for protein content claims; b) discussed current policies related to protein content claims; and c) addressed the pros and cons associated with alternatives to current protein quality assessment methods. Engaging government representatives provided opportunity for informed dialogue and enhanced clarity in the presentation of respective regulatory approaches. The following sections reflect summaries of: 1) The Protein Rating System, used in Canada; 2) The Protein-Digestibility Corrected Amino Acid Score (PDCAAS) system, used in the United States; 3) the Protein Quantity as a percentage of energy system, used in the European Union; and 4) the Digestible Indispensable Amino Acid System (DIAAS), proposed by FAO.

The Protein Rating System: The current regulation of protein content claims in Canada requires determination of a Protein Rating (PR) for a food item, according to the “Official Method FO-1, October 15, 1981”, published by Health Canada (Government of Canada, 1981). Establishing a PR for a food product requires two key components: 1) the availability of an Adjusted Protein Efficiency Ratio (PER); and 2) a value for the Reasonable Daily Intake (RDI). The PER is determined using a rodent bioassay, where the food of interest represents the sole source of dietary protein. Test protein and values are adjusted to a standardized PER value for casein of 2.5. The second component, the RDI, represents the reasonable daily intake of the test food. As per the Food and Drug Regulations (FDR) in Canada, foods seeking a PR must use the RDI values outlined in Schedule K (Government of Canada, 2017), a schedule

¹ Supplementary Figure S1 provides a summary of the pre-workshop survey results

² Supplementary Figure S2 provides an overview of the multi-stakeholder workshop program

that is currently limited to 59 foods, or Reference Amounts/Serving Sizes in the absence of RDI values³. Finally, the PR for a given food item is calculated as the product of the mass (g) of protein within a RDI of the food and the Adjusted PER for the food item.

If the PR of the food product is ≥ 20 , it qualifies for a “source of protein” claim. If the PR is ≥ 40 it qualifies for an “excellent source of protein” or “rich in protein” claim. The FO-1 method was added by name and date to the FDR in 1982, making it the only approved method for supporting protein content claims in Canada. Due to embedment of the method in the FDR, any changes require a regulatory amendment. The PR method, and other methods described below, have several benefits and limitations.⁴

PDCAAS: The Food and Drug Administration (FDA) in the U.S. classifies protein content claims as a subtype of nutrient content claims and requires pre-approval (2017). Protein content can be conveyed through a variety of claim types on food labels, which have different cut-off requirements based on the percent daily value for protein per Reference Amount Customarily Consumed (RACC) provided by the food. For example, an “excellent source of protein” or “rich in protein” claim must provide $\geq 20\%$ of Daily Value for protein per RACC (10 grams). These thresholds are corrected for protein quality, using PDCAAS, to provide a corrected amount of protein per serving. The U.S. and Canadian regulatory frameworks have resulted in inconsistencies in permitted protein content claims.⁵

The PDCAAS is determined using methods outlined in the 1991 FAO/WHO Expert Consultation on Protein Quality Evaluation report (FAO/WHO, 1991). The PDCAAS represents the product of: 1) the percent true fecal nitrogen digestibility (corrected for endogenous nitrogen losses), as measured using a

³ Supplementary Table S1 provides examples of PER and RDI values of certain foods

⁴ Benefits and limitations of different protein quality assessment methods are summarized in Supplementary Table S2

⁵ Supplementary Table S1 provides a comparison of foods permitted to carry protein content claims in Canada and the U.S.

rodent bioassay; and 2) the AA composition of the protein and its score against a reference pattern. Thus, the AA(s) present in the lowest amount relative to a reference requirement pattern determine the AA score. PDCAAS truncates all values to 1.0 (maximum value is 1.0) and values ≤ 1.0 indicate at least one AA is limiting relative to the requirement pattern. The FAO/WHO report (1991) defined the reference pattern as the AA requirement of 2-5 year old school children, and is expressed as mg of indispensable AA per g of protein required. If a food protein is completely digested and absorbed the AA score reflects the inherent limitations of a given protein for productive purposes. Most food proteins, particularly plant-based food proteins, are not completely digestible. A rodent bioassay to measure the true fecal nitrogen digestibility provides an estimate of dietary protein digestibility and absorption.

The PDCAAS is an internationally recognized approach to measuring the quality of dietary protein. Unlike the PER method, PDCAAS has the additional value of accommodating quality assessment of protein blends by using a weighted approach if values exist for individual protein sources they contain.

European Union Approach: The U.S. and Canada's requirement of protein quality values to substantiate claims represents the exception rather than the rule (Lewis, 2012). Other jurisdictions rely primarily on protein content, expressed in either absolute terms or as a percentage of energy. Within the European Union "source of protein" and "high protein" claims are permitted when the protein contributes 12 and 20%, respectively, of the energy value of the food (European Commission, 2006).

DIAAS: Both the PR and PDCAAS approaches require the use of a rodent bioassay. This choice of species, as well as other factors, have been challenged over time, leading to the recommendation for a new approach to assess protein quality: DIAAS (FAO/WHO, 2013). Key recommendations arising from the DIAAS report include that: 1) individual AAs, rather than protein, should be the focus of requirement

estimates and digestibility coefficients; 2) correction for digestibility would be based on ileal AA digestibility coefficients, and not fecal nitrogen digestibility (as used in the PDCAAS model); 3) digestibility coefficients be measured using, in descending order of preference, human subjects, cannulated swine, or rodent assays; and 4) when protein sources are used as ingredients, the DIAAS values for individual proteins would not be truncated to 1.0 before blending, thus ascribing higher values for certain protein ingredients, notably animal-based protein sources. A full description of the assumptions used and the respective justifications are included in the FAO/WHO report (2013).

A Path Forward: Addressing Research and Regulatory Gaps

Harmonization: The Need for a Single, Agreed Upon, Validated Method

Variability across jurisdictions creates barriers to innovation and the development of consistent nutritional messaging. If protein quality assessment is to remain a requirement for protein content claims, the use of one internationally-adopted method for assessment is needed. General consensus, from published reports and workshop attendees (including Health Canada), indicates that the PR method used in Canada is not preferred due to the limitations listed above. Furthermore, the proposed DIAAS approach does not currently meet the needs of regulatory agencies in North America for a number of reasons. These include a lack of: 1) ileal digestibility coefficients for AAs in human foodstuffs; 2) knowledge of the impact of existing and novel food processing methods on ileal digestibility coefficients and AA content for traditional and emerging protein-rich foods; 3) data supporting the DIAAS approach as relevant to nutrition and food policy in North America; and 4) justification for use of invasive laboratory animal experimentation for routine, regulatory purposes.

The above-mentioned challenges are not limited to the DIAAS method, as the existing PDCAAS methodology is hampered by similar concerns, including absence of data on true fecal protein digestibility coefficients for novel processes and foods, and the required usage of rodent-based

bioassays. However, on balance, PDCAAS is the most applicable method and is already used in several countries, notably the U.S. Recently, the Canadian Food Inspection Agency (CFIA) has permitted the use of PDCAAS values to estimate PER ($\text{PDCAAS} \times 2.5 = \text{estimated PER}$) for the establishment of a PR for a given food in Canada. There are limitations to the latter approach as the relationship between PER and PDCAAS is not linear, as they derive from different biological responses. Furthermore, it is not ideal to have some PR values derived from measured PER and others from estimated PER via PDCAAS. Despite the fact that CFIA permits PDCAAS values to estimate PER, if a protein content claim is challenged, measured PER data via the FO-1 method must be provided. Most companies avoid regulatory uncertainty and risk, and therefore utilize the PER method. For Canada to adopt PDCAAS, further amendments would be needed including adoption of a % Daily Value for protein, and associated changes in permitted source claims. Given recent attention to protein-rich foods in the principles guiding the next iteration of Canada's Food Guide, these actions may be timely.

Protein is the only nutrient that requires the use of an animal-based bioassay for the establishment of data to support nutrient content claims. The use of *in vitro* methods would obviate the need to rely on *in vivo* measurements, derived from animal bioassays or, as suggested by the DIAAS report, human subjects. Advancements have been made in the measurement of *in vitro* protein digestibility values, using bench-top, static approaches, leading to rapid and cost-effective approaches to assess factors impacting the ability of a given food protein to yield available AAs (Tinus et al., 2012). Reasonable agreement is achieved between *in vivo* fecal protein digestibility and values obtained using a static, *in vitro* method.⁶ The disadvantage of these methods is they do not precisely mimic human gastrointestinal conditions. Dynamic, computer-controlled gastrointestinal digestion simulation systems have been developed (Havenaar et al., 2016) and permit more precisely controlled digestion conditions,

⁶ Supplementary Figure S3 compares *in vivo* fecal protein digestibility values to *in vitro* values

including transit time, pH and peristaltic mixing of digesta. The dynamic systems, while more reflective of human AA digestion, are expensive, require specialized training to operate, and are available in only a few locales. Efforts should continue to develop and validate *in vitro* methods for the measurement of either protein or AA digestibility. Continued reliance on costly animal testing for regulatory purposes places current policies at odds with societal desires to reduce the usage of animal testing.

Accurate Digestibility Data, Databases and Technical Considerations

Comprehensive international databases housing protein quality values would advance protein quality assessments and their use in regulations. These databases need to be widely accepted by the scientific community and cover a diverse range of foods commonly consumed. Similarly, databases with accurate digestibility scores and AA profiles for foods and mixtures are needed. Protein and true ileal AA digestibility databases, for a wide range of foods and under changing environments and processing conditions, are needed for DIAAS to be a feasible method in the future. This would streamline the calculation of protein quality and create harmonization.

The impact of food processing and anti-nutritional factors in foods are not currently captured in assessment methods (PDCAAS, DIAAS), and pose a barrier in accurately determining protein quality. The interaction of protein-containing foods and ingredients is also an important consideration currently overlooked. As humans typically eat a variety of foods and do not rely on a single protein source, the impact of dietary patterns and complementary proteins is vital in understanding the protein quality of diets. Related to this is the consumption time of multiple protein sources on the quality rating.

Proteins have many functional roles (regulation of physiology and metabolism) beyond providing AAs for protein synthesis, and quality assessment methods often overlook this. Additionally, protein quality and quantity requirements for optimal health varies greatly across life stages and lifestyles (vegetarian, elite athletes). More research is needed to understand the protein needs of

various populations, and how consumers use protein content claims and their impact on food choice and health.

Is There a Human Health Impact from Measuring Protein Quality for Claims?

In developed countries the general adult population has access to an abundance of protein, both in quantity and variety. With adequate protein intakes from a variety of sources, quality may not be a concern for most North Americans. This needs to be assessed by applying a protein quality factor to current dietary survey data of protein intakes. A greater emphasis on quality ratings for proteins may also have unintended consequences for human and environmental health. As vegetable protein sources typically do not qualify for content claims in Canada, it may create a barrier to promotion of non-animal protein foods. Research on the impact of protein quality and quantity ratings on global agriculture, sustainability, and dietary patterns is needed.

While the general North American population may not need protein quality ratings there remain vulnerable populations that rely on high quality protein foods. Those dependent on single protein sources such as infants and those with compromised gastrointestinal function or requiring special dietary products need high quality and easily digested proteins to avoid inadequacy. The PDCAAS/DIAAS methods support this needed evaluation. In addition, food safety is a consideration. New methods for detecting adulterated protein sources (ie: melamine in milk powder) are needed.

Recommendation for Protein Claim Regulation in Canada

There was consensus from workshop participants that PDCAAS is currently the most practical method to measure protein quality for content claims in Canada. Given the desire for harmonization and the advantages of using PDCAAS over PER, and the uncertainty of the timeline for the introduction (if any) of the DIAAS or *in vitro* methods, we recommend that Canada adopt PDCAAS as the accepted method for substantiating protein content claims. Use of PDCAAS will promote innovation and

economic growth within the Canadian and American protein supply chain, harmonize protein messaging across jurisdictions, and serve the population well with little risk. To better enable protein claim regulations to keep pace with rapid advances in analytical methods and best meet the needs of consumers, regulatory tools such as Incorporation by Reference Documents should be explored. Development of *in vitro* methods should continue, and may provide an ethical, quick, cost effective, and accurate measurement of protein/AA digestibility.

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Conflicts of Interest

The authors have no conflicts of interest to report.

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