



Validating An *In Vitro* Method for True Fecal Protein Digestibility Assessment: A Collaborative Study Protocol



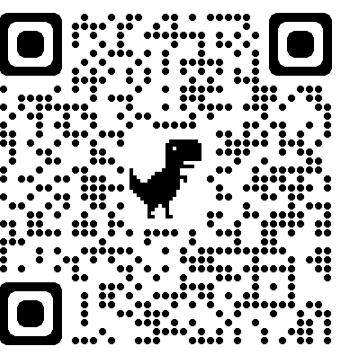
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INTRODUCTION

- **Rodent bioassays** required to establish protein content claims in North America impede protein food innovation and conflict with public opposition to animal testing, hindering novel protein food development.
- Finding **alternative methods** for assessing protein quality without animals is challenging, especially for protein ingredient suppliers and those seeking Vegan Certification
- It is crucial to establish a scientifically valid, and ethically sound approach to assess protein quality, particularly in the **North American context**
- *In vitro* methods were positioned at the FAO/WHO 1991 meeting¹, but **never validated**

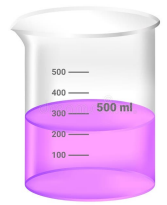


Current *in vivo* method for **Protein quality** evaluation:

- ✓ Protein digestibility-corrected amino acid score (PDCAAS)¹

In vitro methods for **Protein quality** evaluation:

- ✓ pH-drop & pH-stat methods estimate TFPD% in the calculation of PDCAAS, the currently approved method for verifying protein content claims



OBJECTIVES

To validate two static *in vitro* methods – the pH-stat and the pH-drop –for determining TFPD%



- **Recruit** a total of 8 laboratories (4 in Canada, and 4 in the United States)
- Assess **interlaboratory performance** of both methods
- Establish the methods' **equivalence** to established *in vivo* assays
- Provide evidence of alternative *in vitro* methods to assess protein digestibility for **protein content claims**

METHODOLOGY

RECRUITMENT & TRAINING

- 8 labs with demonstrated **experience** with the pH-drop and pH-stat methods were recruited
- Through an iterative process, Official Methods were adapted and **approved** by AOCS
- These methods, along with customized **training videos** were provided to participating laboratories

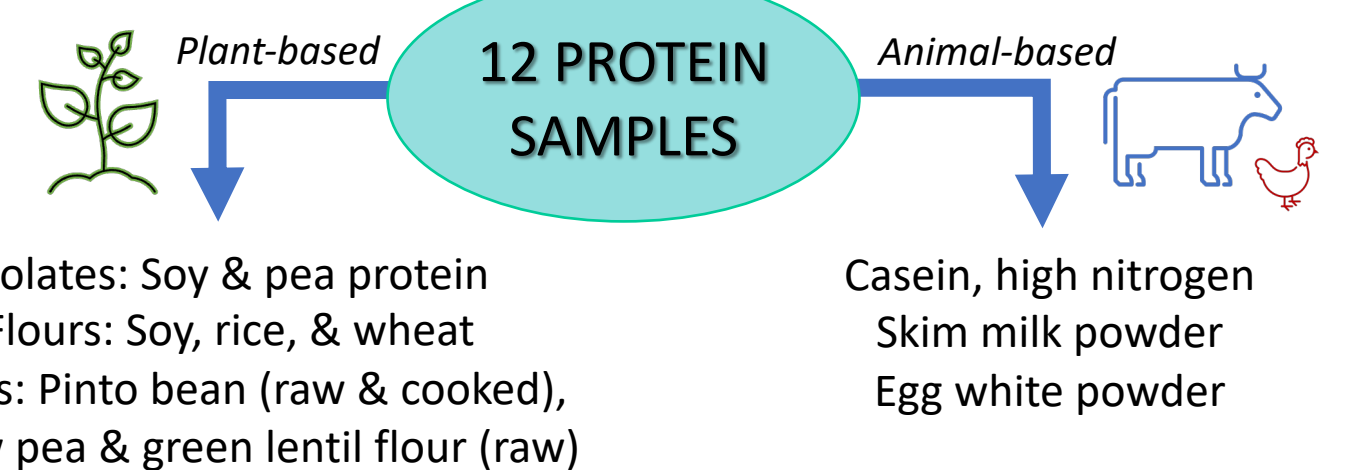


PRE-TRIAL TESTING

- To ensure **consistency** and **accuracy**, two pre-trial samples were provided to participants for testing
- Results from all labs will be analyzed prior to approval to proceed with full testing
- Each participant will receive a report of laboratory results, together with statistical analysis and outlier identification.
- If large deviations from mean values are observed from a lab, the cause will be investigated and remediated

SAMPLE SELECTION

- 12 protein samples were selected based upon availability of published data from ileal amino acid digestibility studies
- All samples were randomly coded, and provided in triplicate to all participating laboratories



EVALUATION

- Results will be **evaluated by AOCS** to determine whether the interlaboratory performance of the methods meet the criteria for approval as **Official Methods**
- Results will be published in an appropriate peer-reviewed journal
- The final AOCS approved method and data will be shared with the **FDA and Health Canada** for consideration as methods that can be used for calculating PDCAAS for use in **protein content claims**

IMPACT

- Promote **innovation** in the development of high-quality foods through **protein content claims** and address **public concerns** surrounding animal research
- Enable a **cost-effective** solution to determine protein digestibility within the **North American context**

STRENGTHS & LIMITATIONS

- There are other more evolved methods for testing protein digestibility (ie. INFOGEST)
- However, the advantage of the pH-drop and pH-stat methods is that they are **rapid, inexpensive**, and can be performed in any laboratory with standard equipment

REFERENCES

- ¹FAO/WHO. Protein Quality Evaluation Report of Joint FAO/WHO Expert Consultation; FAO food and nutrition paper; Food and Agriculture Organization of the United Nations: Rome, Italy, 1991; Vol. 51.
- ²Pedersen, B.; Eggum, B. O. Prediction of Protein Digestibility by an in Vitro Enzymatic pH-Stat Procedure. Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde 1983, 49 (1-5), 265-277.
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- ⁴Tinus, T., Damour, M., Van Riel, V., & Sopade, P. A. (2012). Particle size-starch-protein digestibility relationships in cowpea (Vigna unguiculata). J Food Engineering, 113(2), 254-264.

ACKNOWLEDGEMENTS



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