

Determination of True Ileal Amino Acid Digestibility in the Growing Pig for Calculation of Digestible Indispensable Amino Acid Score (DIAAS)

Suzanne M Hodgkinson,¹ Hans H Stein,² Sonja de Vries,³ Wouter H Hendriks,³ and Paul J Moughan¹

¹Riddet Institute, Massey University, Palmerston North, New Zealand; ²Department of Animal Sciences, University of Illinois, Urbana, IL, USA; and ³Animal Nutrition Group, Wageningen University & Research, Wageningen, The Netherlands

ABSTRACT

Digestible indispensable amino acid score (DIAAS) has been recommended by the FAO for the evaluation of protein quality in human foods, but the application of DIAAS is currently limited because of a lack of published data on the true ileal amino acid (AA) digestibility of AAs in foods. The importance of DIAAS is highlighted. To calculate DIAAS, it is necessary to determine the true ileal AA digestibility of human foods using the growing pig as an animal model for the human based on previous FAO recommendations. A method is described in detail in Supplemental Methods to determine the true ileal AA digestibility of foods for humans using the pig as a model for the adult human. Adoption of the method will enable consistency in the development of databases on predicted true ileal AA digestibility in human foods for the calculation of DIAAS. *J Nutr* 2020;150:2621–2623.

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In 2013, the use of digestible indispensable amino acid score (DIAAS) for evaluating protein quality in ingredients and foods for humans was recommended by the FAO (1, 2). To calculate the DIAAS of a food, it is necessary to have data on the content of true [corrected for gut nondietary amino acids (AAs)] ileal (determined at the end of the small intestine) digestible AAs of the food. For regulatory purposes, DIAAS is determined by comparing concentrations of true ileal digestible AAs, one-on-one, with standard recommended AA requirements for a child (6 mo to 3 y old) expressed as amounts of AA per gram of protein (1). The first-limiting AA defines the DIAAS.

Currently, DIAAS cannot be fully implemented by the industry because there are limited data for the true ileal digestibility of AAs in foods (1, 3–5). Ideally, true ileal AA digestibility coefficients for human foods would be determined directly in humans. However, collecting digesta from the terminal ileum in humans is invasive and not possible on a routine basis. Recently, isotope-based methods for determining AA digestibility in humans have been developed that do not require digesta sampling (4), but these methods have not been

validated in humans to date. Although they offer promise for determining AA digestibility in humans under specific circumstances, these methods are not suitable for routine food evaluation. Moreover, although there are several in vitro models that have been developed to determine true ileal AA digestibility, these have not been validated, and there is no agreement on which of the in vitro methods would be the most appropriate to provide data that could be used to determine DIAAS for human foods. For a review on in vitro methods and their use to predict true ileal AA digestibility, see Wang and Zijlstra (6).

Thus it is necessary to use an animal model. The 2011 FAO Expert Consultation (1) recommended establishing a robust regression relation between human true ileal AA digestibility (adult human ileostomates) and pig true ileal AA digestibility (T-cannulated growing pigs) to allow establishment of a databank of predicted human true ileal AA digestibility values in human foods that have been fed to pigs as they are consumed by humans. This is an important point, in that notwithstanding that the growing pig is a good animal model for protein digestion in humans, the final values to be used in calculating DIAAS are “predicted human” digestibility values—not pig values.

The growing pig is the recommended first-choice animal model for the determination of ileal AA digestibility for the evaluation of protein quality in humans (1, 3) and has been shown to be a valid animal model for this purpose (7–10). In practice, similar ileal AA digestibility coefficients have been determined in the growing pig and adult human for some highly

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Address correspondence to SMH (e-mail: S.M.Hodgkinson@massey.ac.nz).

Abbreviations used: AA, amino acid; DIAAS, digestible indispensable amino acid score; WPI, whey protein isolate.

digestible foods (11, 12). In 2 controlled comparison studies, there was no systematic difference between species for true AA digestibilities. In the work by Rowan et al. (11) working with human ileostomates, the human digestibility values were greater ($P < 0.05$) than those obtained in the growing pig for threonine, tyrosine, phenylalanine, and methionine, whereas no differences between humans and pigs were observed for any other AAs. In the study by Deglaire et al. (12) using nasoileal intubation, true digestibility values for histidine, lysine, phenylalanine, and tyrosine in the pig were slightly greater than in the adult human, but no differences for other indispensable AAs were observed.

Another considerable advantage of the growing pig model is that a very large dataset of true ileal AA digestibility of foods and feedstuffs already exists, which will be readily translatable to the “predicted human” dataset at little extra cost. Nevertheless, the need remains to determine true ileal AA digestibility values for more foods, and especially for foods that are prepared in the same form as consumed by humans. The provision of a database of “predicted human” true ileal AA digestibility values for the world’s foods will allow for an orderly implementation of DIAAS.

In 2014, an FAO Expert Working Group met in Bangalore, India, to discuss methodologies for determining DIAAS values in human foods, and the group published an outline of a method for determining true ileal AA digestibility in the growing pig (4). This protocol, although providing a sound basis for a standardized method is, however, high level and lacks detail around some important considerations. Recently, a major international scientific collaboration (Proteos) has been initiated, aiming to 1) obtain a robust pig/human AA digestibility prediction equation, and 2) populate a large dataset of the AA digestibility of foods as consumed by humans. In preparation for this work, scientists and veterinary surgeons from the Riddet Institute (New Zealand), University of Illinois (USA), and Wageningen University & Research (The Netherlands) worked together to generate a detailed description of an appropriate method for use in the pig to determine the true ileal AA digestibility of foods. The latter protocol is based on the Bangalore Working Group recommendations (4), but provides those working in the field with more detailed information needed to conduct the digestibility assay. The fully developed protocol is provided as **Supplemental Methods**, with the intention that scientists adopt the protocol more generally, thus providing for consistency in the generation of DIAAS values.

The protocol described was developed to assist different laboratories in determining values for DIAAS using a standard method, to maximize consistency in food evaluation. This follows the recommendations of FAO (1). Thus data can be generated in different laboratories for the true ileal AA digestibility of a wide range of foods. The generation of these data will remove the current limitation on the use of DIAAS by industry, in accordance with the recommendations of FAO Expert Consultations and Working Groups (1, 3, 4).

To give more information about the conditions used to determine DIAAS values, it is recommended that scientific publications include information such as the breed of the pigs used, their body weight during the test period, and any significant food refusals. Also, any health problems that occurred during the test period should be described. Other than immediately following surgery (as described in the protocol), pigs that require treatment with antibiotics should be excluded.

One factor that was not described in the FAO publication (4) was the gender of the pigs to be used. Male pigs are often

used for these types of assays, but the decision was made that it would be better to use female pigs. The reason for this is that in some countries male pigs are castrated at a very young age. Differences have been demonstrated between entire and castrated males in terms of factors such as growth rate and nitrogen balance in the 30–100-kg live weight range (13), which could reflect small differences in nutrient digestibility. The use of female pigs allows better standardization. Given that gilts from modern genotypes do not reach puberty until they are >100 kg body weight (unless they are exposed to boars), the assays will be completed before the females reach puberty.

It is important that the test foods are fed to pigs in a form that is as close as possible to the form the foods are consumed by humans. This includes particle size and preparation/cooking conditions. In pigs it is well known that particle size impacts ileal digestibility, with a decrease in particle size increasing apparent ileal digestibility values (14). In addition, heat treatments applied to food can lead to changes in AA digestibility and bioavailability values compared with the same, untreated food (15, 16). An advantage of testing the foods in the pig, as opposed to other species such as the rat, is that the pig readily consumes foods in a range of formats, whereas the rat is a selective eater: foods must be ground and mixed with other components in the diet to ensure the rat does not select only 1 part of the diet. As previously stated, grinding the food could affect its digestibility. When the food is composed of larger particles, such as peanuts and many legumes, the food might need to be broken up to mix it well with the indigestible marker. Marker homogeneity is an essential aspect for accurate estimation of nutrient digestibilities. If the food is broken up or crushed, the degree to which this is done must be reported. The food should not, however, be ground, unless the ground form is what is consumed by humans.

If there are several test proteins containing <10% protein, the pig is likely to enter a negative nitrogen balance, which could affect the results obtained. To avoid this, it is recommended that ≤ 2 diets containing <10% protein be fed sequentially. If several diets containing <10% protein are to be tested, the diets should either be supplemented with synthetic AAs during days 1–3 of the 7-d period, or the basal diet should be fed for 3–7 d between diets containing the test proteins.

The pig digestibility value is an input to a regression equation, allowing prediction of true ileal AA digestibility for the food. The currently available equation (12) for total nitrogen (N) is $y = 1.47x - 0.47$ ($R^2 = 0.88$), where x is true N digestibility in the pig, and y is the predicted value for true N digestibility in the human. Completion of the Proteos project will, however, provide a prediction equation based on a greater number of foods, with a larger range of true ileal AA digestibilities.

As well as being used to calculate DIAAS, the true ileal AA digestibility values determined using this protocol can also be used to evaluate combinations of food sources based on their full digestible AA profile. This can be used both for human and pig nutrition.

The protocol has been developed by an international team of collaborators, and the procedures have been chosen carefully to allow for accuracy, practicality, and ready duplication. The protocol has been successfully applied at the Riddet Institute, Massey University, New Zealand; the University of Illinois, Urbana-Champaign, USA; and at Wageningen University & Research, The Netherlands. In all cases, the pigs have remained healthy throughout the studies and have tolerated the cannula with no apparent problems. Digesta collections have been

successful, and the results obtained have been within expected parameters. The repeatability and reproducibility for the determination of true ileal AA digestibility coefficients have been evaluated for the well-characterized protein whey protein isolate (WPI) between and within the 3 laboratories using the protocol described in Supplemental Methods (data not reported here). The CV values for the indispensable AAs (the values used to calculate DIAAS) averaged 1.8% and varied from 0.01% (reactive lysine) to 3.2% (threonine) between laboratories. Within laboratories, the CV for the indispensable AAs averaged 1.1% and ranged from 0.01% (methionine) to 10.3% (threonine). Collaborative studies only considering AA analysis have given CV values between 4% and 20% (17), so the variation in values obtained for WPI can be considered to be low, supporting the repeatability and reproducibility of the assay.

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References

1. Food and Agriculture Organization of the United Nations (FAO). Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. Rome: FAO; 2013.
2. Moughan PJ. Advances in describing dietary protein quality for humans. *J AOAC Int* 2020. In press.
3. Food and Agriculture Organization of the United Nations (FAO). Protein quality assessment in follow-up formula for young children and ready to use therapeutic foods. Rome (Italy): FAO; 2018.
4. Food and Agriculture Organization of the United Nations (FAO). Research approaches and methods for evaluating the protein quality of human foods. Report of a FAO Working Group. Rome: FAO; 2014.
5. Codex Alimentarius Commission FAO/WHO. Joint FAO/WHO Food Standards Programme. Codex Committee on Nutrition and Foods

- for Special Dietary Uses. Proposed draft guideline for ready-to-use therapeutic foods. Rome (Italy): FAO/WHO; 2016.
6. Wang LF, Zijlstra RT. Prediction of bioavailable nutrients and energy. In: Moughan PJ, Hendriks WH, editors. Feed evaluation science. Wageningen (The Netherlands): Wageningen Academic Publishers; 2018. pp. 337–86.
7. Deglaire A, Moughan PJ. Animal models for determining amino acid digestibility in humans – a review. *Br J Nutr* 2012;108:S273–S81.
8. Moughan PJ, Birtles MJ, Cranwell PD, Smith WC, Pedraza M. The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. *World Rev Nutr Diet* 1992;67:40–113.
9. Moughan PJ, Cranwell PD, Darragh AJ, Rowan AM. The domestic pig as a model animal for studying digestion in humans. In: Souffrant W-B, Hagemester H, editors. VIth International Symposium on Digestive Physiology in Pigs; 1994 1994. European Association of Animal Production; 1994. pp. 389–96.
10. Moughan PJ, Rowan AM. The pig as a model animal for human-nutrition research. In: Savage GP, editor. Proceedings of the Nutrition Society of New Zealand. Vol. 14. Canterbury: Nutrition Society of New Zealand; 1989. pp. 116–23.
11. Rowan AM, Moughan PJ, Wilson MN, Maher K, Tasman-Jones C. Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. *Br J Nutr* 1994;71:29–42.
12. Deglaire A, Bos C, Tome D, Moughan PJ. Ileal digestibility of dietary protein in the growing pig and adult human. *Br J Nutr* 2009;102:1752–9.
13. Elsbernd AJ, Stalder KJ, Karriker LA, Patience JF. Comparison among gilts, physical castrates, entire males, and immunological castrates in terms of growth performance, nitrogen and phosphorus retention, and carcass fat iodine value. *J Anim Sci* 2015;93:5702–10.
14. Flis M, Sobotka W, Purwin C. Effect of feed structure on nutrient digestibility, growth performance, and gastrointestinal tract of pigs – a review. *Ann Anim Sci* 2014;14:757–68.
15. Almeida FN, Htoo JK, Thomson J, Stein HH. Amino acid digestibility of heat damaged distillers dried grains with solubles fed to pigs. *J Anim Sci Biotechnol* 2013;4:44.
16. Rutherford SM, Moughan PJ. Application of a new method for determining digestible reactive lysine to variably heated protein sources. *J Agric Food Chem* 1997;45:1582–6.
17. Rutherford SM, Moughan PJ. Principles of chemical analysis. In: Moughan PJ, Hendriks WH, editors. Feed evaluation science. Wageningen (The Netherlands): Wageningen Academic Publishers; 2018. pp. 21–37.