NRC Research Press

REVIEW

# Comparison of methodologies used to define the protein quality of human foods and support regulatory claims

Wilfredo D. Mansilla, Christopher P.F. Marinangeli, Cara Cargo-Froom, Adam Franczyk, James D. House, Rajavel Elango, Daniel A. Columbus, Elijah Kiarie, Mike Rogers, and Anna K. Shoveller



**Abstract:** Protein quality (PQ) is the capacity of a protein to meet the amino acid (AA) requirements of an individual. There are several methodologies for determining the PQ of foods. The protein efficiency ratio is an animal growth bioassay. The protein-digestibility-corrected AA score considers the AA requirements of a reference population, and the true nitrogen digestibility coefficient for each ingredient. The digestible indispensable AA score is based on true ileal AA digestibility and better represents bioavailability of AAs. In vitro techniques for assessment of PQ are available but require validation against a greater range of protein sources. Isotopic methods, such as the indicator AA oxidation and dual tracer techniques measure AA relative bioavailability and digestibility, respectively, but require sophisticated equipment, and may not be cost nor time effective for the industry to adopt. The present review discusses advantages and disadvantages of methodologies for determining PQ of food for humans focused on methods that are or could be adopted by regulatory agencies. Understanding the framework and resources available for PQ determination will help in the selection of appropriate methods depending on the application.

**Novelty**

- Understanding the framework and resources available for PQ determination will help in the selection of appropriate methods depending on the application.

*Key words:* amino acid, bioavailability, DIAAS, digestibility, indicator amino acid oxidation, PDCAAS, PER, protein, protein quality, in vitro.

**Résumé :** La qualité d'une protéine (PQ) se mesure à sa capacité de répondre aux besoins en acides aminés (AA) d'un individu. Il existe plusieurs méthodologies pour déterminer la PQ des aliments. Le coefficient d'efficacité protéique (PER) est un essai biologique de croissance animale. Le score AA corrigé de la digestibilité des protéines (PDCAAS) tient compte des besoins en AA d'une population de référence et du véritable coefficient de digestibilité de l'azote pour chaque ingrédient. Le score de digestibilité des AA essentiels (DIAAS) est basé sur la véritable digestibilité iléale des AA et représente mieux la biodisponibilité des AA. Des techniques in vitro pour évaluer la PQ sont disponibles, mais nécessitent une validation par rapport à une plus grande gamme de sources de protéines. Les méthodes isotopiques telles que l'indicateur de l'oxydation des AA et les techniques de double traceur mesurent respectivement la biodisponibilité et la digestibilité relatives des AA, mais nécessitent un équipement sophistiqué et peuvent ne pas être rentables ni efficaces en termes de temps pour que l'industrie les adopte. La présente analyse documentaire examine les avantages et les inconvénients des méthodologies de détermination de la PQ des aliments pour l'humain axée sur les méthodes qui sont ou pourraient être adoptées par les organismes de réglementation. La compréhension du cadre et des ressources disponibles pour la détermination de la qualité des protéines aidera à sélectionner les méthodes appropriées en fonction de l'application. [Traduit par la Rédaction]

**Nouveauté**

- La compréhension du cadre et des ressources disponibles pour la détermination de la qualité des protéines aidera à sélectionner les méthodes appropriées en fonction de l'application.

*Mots-clés :* acide aminé, biodisponibilité, DIAAS, digestibilité, indicateur d'oxydation des acides aminés, PDCAAS, PER, protéine, qualité des protéines, in vitro.

Received 12 October 2019. Accepted 26 March 2020.

**W.D. Mansilla, C. Cargo-Froom, E. Kiarie, and A.K. Shoveller.*** Department of Animal Biosciences, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada.
**C.P.F. Marinangeli.** Pulse Canada, 920-220 Portage Avenue, Winnipeg, MB R3C 0A5, Canada.
**A. Franczyk and J.D. House.** Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada.
**R. Elango.** Department of Food and Nutritional Sciences, School of Population and Public Health, The University of British Columbia and BC Children's Hospital Research Institute, 950 West 28th Ave., Vancouver, BC V5Z 4H4, Canada.
**D.A. Columbus.** Prairie Swine Centre, Inc, 2105 – 8th Street East, Saskatoon, SK S7H 5N9, Canada.
**M. Rogers.** Department of Food Science, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada.

**Corresponding author:** Anna K. Shoveller (email: ashovell@uoguelph.ca).

*A.K. Shoveller currently serves as an Associate Editor; peer review and editorial decisions regarding this manuscript were handled by Daniel Moore and Wendy Ward.

## Introduction

All animals, including humans, require an exogenous source of indispensable amino acids (IAAs). Dietary IAAs consumed as intact protein are required for meeting daily amino acid (AA) requirements for body functions (e.g., lean tissue maintenance, growth, milk production, reproduction). The ability of a food source (e.g., mixture of proteins) to satisfy an individual's IAA requirements is a reflection of protein quality (PQ) (Schaafsma 2005; Nosworthy and House 2017). Proteins are digested to liberate AAs for absorption in a suitable form for maintenance and (or) growth. This concept of AA bioavailability (Batterham 1992) is fundamental to the determination of PQ. Food processing, cooking (e.g., extrusion or fermentation), or storage conditions can alter digestibility and bioavailability of AAs and affect PQ (Khattab et al. 2009; Sarwar 1997). PQ not only depends on the characteristics of the ingredient that is consumed, but also on the physiology of the individual consuming the food (Nosworthy and House 2017; Schaafsma 2005). Moreover, within the same species, the IAA requirements differ across and within age and physiological stages (FAO 2013; NRC 2012) and can result in a variety of PQ values for the same ingredient. Thus, measured PQ is not a fixed property of a food or its ingredients and can be affected by multiple factors, including food matrix, processing/cooking conditions, the physiological AA requirements of species consuming the food (Table 1), as well as other physiological factors (e.g., growth).

From a dietary perspective, there is no requirement for protein per se, but rather a requirement for the IAAs that compose the protein (Elango et al. 2012; Hoffer 2016; Schaafsma 2005). Of the 20 common AAs needed for the synthesis of mammalian proteins, 9 are considered indispensable for adult humans (FAO 2013) and defined as those for which the individual is strictly dependent on a dietary supply to meet their daily needs. In a specific protein or food (mix of proteins), the IAA present in the lowest proportion relative to the IAA pattern requirement is referred to as the limiting AA and derived from the ideal protein concept where the relative proportion of available IAAs is similar to IAA requirements of the individual. Given that the ability to assimilate AAs for metabolic work is dependent on the availability of the limiting IAA, the limiting IAA determines the rate of utilization of all other bioavailable AAs (Fuller et al. 1989). Thus, the PQ of a food is directly related to the limiting AA in that individual or population.

The concept of PQ is not commonly used among animal nutritionists as often as by human nutritionists. Animal nutritionists formulate single source diets for agricultural and companion animals and, therefore, tend to focus on the levels of bioavailable AAs, not PQ. In contrast, human nutritionists cannot control the dietary habits of the individual or population. The PQ concept is used to facilitate guidance on sources of protein for the consumer. Because of the breadth of foods that humans consume, criteria have been developed by the FAO to capture the scientific criteria for measuring PQ. For the purpose of this review, we focus on methods that are cost effective, minimally invasive, and validated in animals and (or) humans.

There are a number of methodologies for determining PQ of foods. The protein efficiency ratio (PER) compares the mass gained by growing rats that consume a diet with the test protein or a casein-based diet (standard protein) (Marinangeli and House 2017). PER is similar to net protein utilization (NPU) and is calculated on the basis of nitrogen retention. The protein-digestibility-corrected AA score (PDCAAS) and the digestible IAA score (DIAAS) use IAA requirements of a reference population and depend on true total tract fecal protein digestibility and true ileal AA digestibility, respectively (FAO 2013). More sophisticated methodologies in humans, such as the indicator AA oxidation (IAAO) technique, net post-prandial protein utilization (NPPU), dual stable isotope tracer approach and the in vitro TNO gastro-intestinal model (TIM-1), have recently become options for measuring the PQ of a food or ingredient (Humayun et al. 2007; Minekus et al. 1995). The objective of this review is to describe and compare the aforementioned methodologies for determining AA bioavailability and PQ of food and context for supporting regulations that could be readily adopted by the food industry.

## Growth-based method: the PER

The PER was described by Osborne et al. (1919) and was the first method developed for assessing PQ for human foods (FAO 2013). The PER method calculates the efficiency of utilizing dietary protein for body weight gain (e.g., growth). The test and reference protein sources are incorporated into the diets at the same relative proportion of the total diet by mass. These proteins are not necessarily limiting in the same IAAs. Casein is, by convention, the preferred reference protein for PER, but other high-quality proteins could serve as the reference protein. The use of casein allows the PER method to rank proteins against a single reference, but this ranking is not proportional. A protein with a PER of 2 is not 2× the quality of a food with a PER of 1. The PER is carried out over 28 days for rats, or 14 days for chickens (Boling-Frankenbach et al. 2001; Health Canada 1981). In Canada, the rat model is the official method for determining PQ of human foods and used to confirm the quality of protein in infant formulas in both Canada and the United States of America (FDA 2018). Guidance for measuring the PER on new foods has been outlined by the Canadian government (Government of Canada 1981; Supplementary Fig. S1[1]).

The PER requires that rapidly growing animals, such as weanling rats or 1-week-old chicks, be used over a short period of time to maximize the effect of protein consumption (Buamah and Singsen 1975) and avoid variation in AA requirements due to age. The variation found in the daily requirement (milligrams per day) is not consistent among IAAs (Sakomura et al. 2015). Using older rats (i.e., 2 vs. 1 week of age) has yielded variable results in PER values (Chapman et al. 1959) and the digestibility coefficient for the same protein sources can change in chickens within the first week after hatching (Batal and Parsons 2002; Noy and Sklan 1995). Hence, the digestive capacity and AA requirements of the animals used to generate PER should be recognized.

During growth, AA requirements are expressed as a percentage of dry matter intake and are higher than those required in adulthood (Table 1). These greater requirements can result in an underestimation of PQ when these values are used to quantify the PQ of foods consumed by adults. Moreover, given that the PER is a growth assay, it does not fully credit AAs used for maintenance. During the fastest growth stage, AA requirements for maintenance represent ~10% of the total AA requirement for maximal growth (Moughan 1995), and the proportion used for maintenance increases as the animal matures.

The animal model used to determine PER of a food will also affect the PQ measurements. Sulfur AA (methionine and cyste(i)ne) requirements for rats are much higher than those of pigs, chickens, and humans (Table 2) (FAO 2013; NRC 1995, 2012; Ross 2014). Therefore, when rats are used to measure PQ of human foods, the higher sulfur AA requirements will generate a lower PER for protein sources with low levels of sulfur AAs (milligrams per gram of protein) (e.g., legumes). Similarly, while arginine (Arg) requirements in chickens are nearly double the requirements of rats and pigs, Arg is not an IAA for humans (FAO 2013). Although the growing rat is the preferred method for calculating the PER of human

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/apnm-2019-0757.

**Table 1.** Total AA requirements (mg/g CP) of mammals based on age and (or) stage of development.

| Age/development group | | Aromatic AA | His | Ile | Leu | Lys | Sulfur AA | Thr | Trp | Val |
|---|---|---|---|---|---|---|---|---|---|---|
| **Early growth** | | | | | | | | | | |
| Human[a] | 0.5 years | 52 | 20 | 32 | 66 | 57 | 27 | 31 | 8.5 | 43 |
| Layer chicken[b] | 0–6 weeks | 55.6 | 14.4 | 33.3 | 61.1 | 47.2 | 34.4 | 37.8 | 9.4 | 34.4 |
| Broiler chicken[b] | 0–3 weeks | 39.1 | 15.2 | 34.8 | 52.2 | 47.8 | 39.1 | 34.8 | 8.7 | 39.1 |
| Swine[c] | 5–7 kg | 61.5 | 22.3 | 33.8 | 65.8 | 65.4 | 36.9 | 40.4 | 10.8 | 42.3 |
| Dog[d] | 4–14 weeks | 57.8 | 17.2 | 28.9 | 57.2 | 38.9 | 31.1 | 36.1 | 10.0 | 30.0 |
| **Growth** | | | | | | | | | | |
| Human[a] | 1–2 years | 46 | 18 | 31 | 63 | 52 | 25 | 27 | 7 | 41 |
| | 3–14 years | 41 | 16 | 30 | 61 | 48 | 23 | 25 | 6.6 | 40 |
| | 15–18 years | 40 | 16 | 30 | 60 | 47 | 23 | 24 | 6.3 | 40 |
| Layer chicken[b] | 6–12 weeks | 51.9 | 13.8 | 31.3 | 53.1 | 37.5 | 32.5 | 35.6 | 8.8 | 32.5 |
| | 12–18 weeks | 44.7 | 11.3 | 26.7 | 46.7 | 30.0 | 28.0 | 24.7 | 7.3 | 27.3 |
| | 18 weeks to 1st lay | 44.1 | 11.8 | 26.5 | 47.1 | 30.6 | 27.6 | 27.6 | 7.1 | 27.1 |
| Broiler chicken[b] | 3–6 weeks | 36.0 | 16.0 | 36.5 | 54.5 | 50.0 | 36.0 | 37.0 | 8.0 | 41.0 |
| | 6–8 weeks | 33.3 | 15.0 | 34.4 | 51.7 | 47.2 | 33.3 | 37.8 | 8.9 | 38.9 |
| Rat[e] | ~4 weeks to 6 months | 68.0 | 18.7 | 41.3 | 71.3 | 61.3 | 65.3 | 41.3 | 13.3 | 49.3 |
| Swine[c] | 7–11 kg | 60.8 | 22.4 | 33.3 | 65.0 | 64.6 | 36.7 | 40.1 | 10.5 | 42.2 |
| | 11–25 kg | 63.2 | 23.0 | 34.9 | 67.5 | 67.0 | 37.8 | 41.6 | 11.0 | 43.5 |
| | 25–50 kg | 60.0 | 21.7 | 32.8 | 62.8 | 62.2 | 36.1 | 40.0 | 10.6 | 41.7 |
| | 50–75 kg | 60.6 | 21.9 | 33.5 | 63.2 | 62.6 | 36.8 | 41.3 | 11.0 | 41.9 |
| | 75–100 kg | 62.1 | 22.7 | 34.1 | 64.4 | 63.6 | 37.9 | 42.4 | 11.4 | 43.2 |
| | 100–135 kg | 53.0 | 18.9 | 29.5 | 53.8 | 53.8 | 32.6 | 37.1 | 9.8 | 37.1 |
| Dog[d] | >14 weeks | 57.1 | 14.3 | 28.6 | 46.4 | 40.0 | 30.0 | 35.7 | 10.0 | 32.1 |
| **Maintenance/maturity** | | | | | | | | | | |
| Human[a] | >18 years | 38 | 15 | 30 | 59 | 45 | 22 | 23 | 6 | 39 |
| Layer chicken[b] | 1st lay | 55.2 | 11.2 | 43.2 | 54.4 | 46.4 | 38.4 | 31.2 | 10.4 | 46.4 |
| Rat[e] | Adult | 38.0 | 16.0 | 62.0 | 36.0 | 22.0 | 46.0 | 36.0 | 10.0 | 46.0 |
| Dog[d] | Adult | 42.5 | 18.75 | 37.5 | 67.5 | 35 | 65 | 42.5 | 13.75 | 48.75 |

**Note:** AA, amino acid.
[a]Adapted from FAO (2013).
[b]Adapted from NRC (1994).
[c]Adapted from NRC (2012).
[d]Adapted from NRC (2006).
[e]Adapted from NRC (1995).

**Table 2.** IAA:Lys requirement ratios of different species during the growing stages.

| IAA | Rat[a] | Pig[b] | Chicken[c] | Human[d] | Dog[e] |
|---|---|---|---|---|---|
| Arginine | 47 | 46 | 107 | — | 90 |
| Aromatic AA[f] | 111 | 94 | — | 91 | 149 |
| Histidine | 30 | 35 | — | 35 | 44 |
| Isoleucine | 67 | 52 | 68 | 56 | 74 |
| Leucine | 116 | 101 | 110 | 116 | 147 |
| Lysine | 100 | 100 | 100 | 100 | 100 |
| Sulfur AA[g] | 107 | 56 | 76 | 47 | 80 |
| Threonine | 67 | 60 | 67 | 56 | 93 |
| Tryptophan | 22 | 17 | 16 | 15 | 26 |
| Valine | 80 | 65 | 76 | 75 | 77 |

**Note:** Requirements are presented in relation to Lys; units for expressing amino acid (AA) requirements vary depending on the nutritional knowledge and the diet consumption and behaviour of the animals (meal or ad libitum). IAA, indispensable AA.
[a]Requirements for the growth phase as specified by NRC (1995).
[b]Standardized ileal digestible AA requirements for pigs (25–50 kg) as specified by NRC (2012).
[c]Standardized ileal digestible AA requirements for chickens (11–24 days) as specified by Ross (2014) for Ross 708 broilers.
[d]AA requirements for humans (0.5 months to 3 years) according to FAO (2013).
[e]Requirements for the growth phase of a puppy (4–14 weeks) as specified by NRC (2006).
[f]Aromatic AA: phenylalanine and tyrosine.
[g]Sulfur AA: cysteine and methionine.

foods, PER values should be evaluated before considering them as an accurate representation of PQ, especially for human adults.

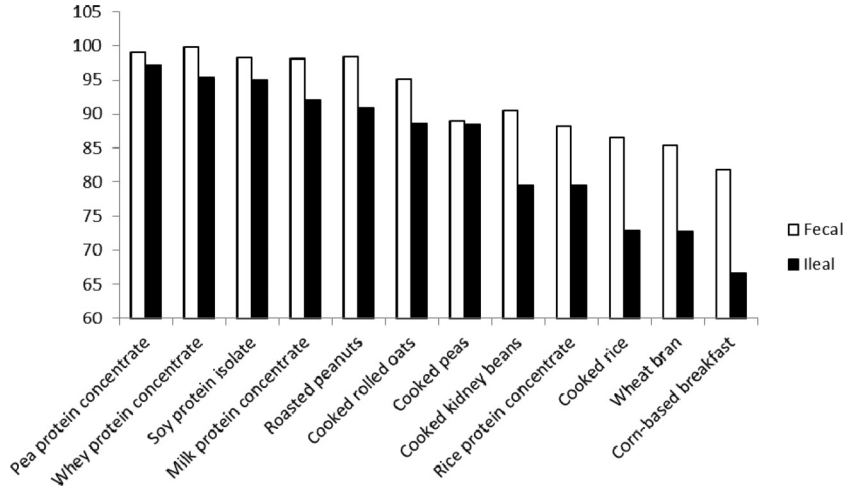
The reference protein for the PER method is casein. The AA content of casein is relatively constant (Beach et al. 1941; Williamson 1944), and the casein control must be conducted in parallel to the test protein rather than using a known value for casein. The final PER value is adjusted by standardizing against the average PER for casein of 2.5 (Government of Canada 1981). Proteins with a PER that is close to or higher than casein are considered to be high-quality protein sources.

The PER method assumes linearity of protein efficiency across different levels of protein intake and among all ingredients compared. This assumption is important as sensorial perception of the test protein by the animal may influence consumption, with both under- and over-consumption resulting in higher or lower PER values compared with when the optimal level of protein is consumed.

The PER of a food is relatively straightforward to generate, but values of ingredients are not additive in mixed meals and require that an animal trial be conducted for every new food or diet mixture produced (Marinangeli and House 2017). The use of growing animals, their different AA requirements compared with humans, and the inability to credit dietary AA used for maintenance render the PER method an incomplete representation of the PQ of foods for humans. The validity of the PER is improved when assessing the PQ for human foods when the diet consists of a sole food source (0–0.5 years old) and during periods of rapid growth (also 0–0.5 years old). However, the flaws associated with differences in requirements between the animal model and humans are acknowledged.

Similar to PER, NPU measures the retention of nitrogen consumed by subtracting nitrogen in the feces and urine from intake and representing that as a percentage of intake (fecal and urine nitrogen excretion/nitrogen intake). NPU is also determined by comparing the test protein to a casein-based control. Animals are fed nitrogen-free diets to determine endogenous losses and this concept is discussed in the ileal digestibility section below. NPU

**Fig. 1.** Fecal and ileal digestibility (true nitrogen digestibility, %) of different human-consumed ingredients determined in growing male rats fed a basal nitrogen-free diet supplemented with the test ingredients as the sole source of protein. Adapted from Rutherfurd et al. (2015).




can be used in adult humans to measure PQ, but subjects need to be well matched and, as such, the use of experimental animals is preferred. We are unaware of any jurisdiction that currently uses NPU to substantiate the PQ of food for humans.

## Determining the digestibility of protein and AAs

Digestibility is a measure of the non-recovery of nutrients at different parts of the digestive tract. Depending on the method, measurements are taken from different locations within the digestive tract and expressed as a proportion of nutrient intake (Stein et al. 2007). It is assumed that the disappearance of nutrients is related to the absorptive capacity in the gastrointestinal tract. However, it may not always align with nutrient bioavailability (Batterham 1992). Digestibility is often used to estimate AA bioavailability (NRC 2012; Stein et al. 2007).

### Apparent and true total gastrointestinal tract digestibility

Apparent total tract digestibility is the proportion of nutrient intake not recovered in feces. For protein, this is also referred to as total nitrogen digestibility, and measures fecal nitrogen recovery relative to nitrogen intake. This method assumes that AAs are digested and absorbed within all segments of the digestive tract (Stein et al. 2007) and can have implications on accuracy of the digestibility coefficients generated. First, no significant absorption of AAs occurs in the large intestine of rats, pigs, chickens, and humans. Different proteins and AAs infused into the large intestine of various monogastric animals, including pigs and humans (Columbus et al. 2014; Darragh et al. 1994; Fuller and Reeds 1998; Fuller 2012; Mason et al. 1976; Mason and Palmer 1973; Webb 1990), significantly increases fecal nitrogen excretion, suggesting that nitrogen, but little AA and protein, are absorbed in the large intestine. Second, the capacity for nitrogen absorption in the large intestine is significant. Nitrogen (protein-, AA-, or non-protein-nitrogen) infused into the large intestine is readily absorbed, with the majority excreted in the urine; mainly as urea (Fuller and Reeds 1998; Just et al. 1981; Krawielitzki et al. 1990; Zebrowska 1975). Nitrogen absorbed in the large intestine is only efficiently assimilated when total nitrogen (i.e., dispensable AA content) is limiting nutrient in the diet (Mansilla et al. 2015). Third, the microbiome has the capacity to modify the AA profile of digesta by catabolizing and synthesizing AAs (Low 1980; Rerat 1981; Zebrowska and Buraczewski 1998). Threonine and tryptophan are usually degraded to a greater extent than other IAAs, and there may be net synthesis of methionine and lysine (Just et al. 1981; Moughan et al. 2014; Tanksley and Knabe 1993). However, IAA synthesis in the large intestine, and subsequent absorption are not necessarily nutritionally relevant or available for protein synthesis in humans (van der Wielen et al. 2017). Furthermore, dietary fibre can increase microbial activity and increase nitrogen levels from non-dietary sources (Jensen and Jørgensen 1994). An estimated 25%–54% of nitrogen entering the large intestine is of dietary origin. However, 80%–90% of fecal nitrogen output represents bacterial protein (Mason et al. 1976; Mason and Palmer 1973). Rutherfurd et al. (2015) determined total tract or ileal digestibility of protein of different ingredients in the rat (Fig. 1) and reported that while there was little impact on fecal digestibility, ileal digestibility was affected by the quality of the ingredient. Similar results have been reported in chickens, pigs, dogs, and humans (Hendriks et al. 2012; Moughan and Donkoh 1991; Murray et al. 1997). To improve the accuracy of total tract protein digestibility coefficients, true fecal nitrogen digestibility can be calculated by correcting for endogenous losses; and used for PQ assessments, but this does not account for nitrogen or AAs absorbed in the large intestine.

### Ileal digestibility and endogenous loses

Given the assumptions made when calculating true total tract protein digestibility, ileal digestibility (i.e., digestibility up to the terminal ileum) has been developed and adopted by the swine and poultry industries for feed formulation. The FAO (2013) has acknowledged that ileal digestibility is more representative of the bioavailability of dietary AAs. However, the lack of published data available in humans has challenged a transition from true tract protein digestibility to ileal AA digestibility when determining the PQ of foods, but a greater amount of ileal digestibility work to calculate DIAAS has been more recently conducted.

Ileal digestibility coefficients better predict growth of animals as the problems associated with microbial protein degradation and disappearance of nitrogen in the large intestine are removed (Just et al. 1985; Low et al. 1982). Studies have reported bacterial AA catabolism in the small intestine (Dierick et al. 1986; Jensen and Jørgensen 1994; Libao-Mercado et al. 2009) and the effects on the accuracy of ileal digestibility values. However, these contributions are minimal compared with nitrogen or AA disappearance in the large intestine (Darragh and Hodgkinson 2000).

Despite being a more accurate representation of protein digestibility, ileal digestibility also presents several methodological challenges (Fuller 2012; Nyachoti et al. 1997; Zebrowska and Buraczewski 1998). First, collection of the digesta is an invasive procedure. In the pig, the most widely used surgical technique is the placement of a T-cannula before the ileocecal valve (Sauer and de Lange 1992). Complete collection of ileal digesta is not possible and an indigestible marker (e.g., titanium dioxide or chromic

oxide) is included for calculation of the total digesta flow (Sauer and Ozimek 1986). The inherent assumptions associated with using indigestible markers and incomplete digesta sampling are required (Adeola 2001). Sampling of ileal digesta from humans is possible in ileostomy patients (Rowan et al. 1994), but the surgical conditions (Dowsett et al. 1990) and the fact that patients often have underlying health issues remain as potential drawbacks.

Second, similar to material collected from the large intestine, AAs in the ileal digesta are not exclusively of dietary origin. Endogenous secretions and microbially synthesized AAs contribute to AAs in the ileal digesta (de Lange et al. 1989; Nyachoti et al. 1997). Endogenous losses of non-dietary AAs can contribute more than 50% of the total ileal AA outflow when feeding high-fibre ingredients (Schulze et al. 1994; Souffrant 2001). Not accounting for endogenous AA losses is the apparent ileal digestibility and can significantly affect estimates of digestibility, especially at low dietary protein intakes (Columbus and de Lange 2012). Accounting for both basal and ingredient-specific endogenous AA losses determines true ileal digestibility and can be estimated using specialized methods, such as protein-free diets or AA-defined diets, and isotopic tracers or markers (Nyachoti et al. 1997).

Third, although true ileal digestibility is considered the most accurate estimate of digestibility, it can also overestimate bioavailability. This overestimation occurs when heat-treated ingredients are used in a diet or when ingredients are stored for long periods. Lysine, a common first-limiting AA from some dietary sources (e.g., cereals), can react with reducing sugars in carbohydrates in the presence of heat (referred to as Maillard reaction; Fernandez and Parsons 1996; Gonzalez-Vega et al. 2011). This Maillard complex can be absorbed, but bound lysine cannot be used by peripheral tissues for protein synthesis, not affecting digestibility, but reducing bioavailability of AAs.

Because ileal digestibility can be a challenging and invasive method, pigs are often considered an excellent model for the digestive capacity and metabolism of AAs in humans (Miller and Ullrey 1987; Moughan and Rowan 1989). Pigs are monogastric, omnivorous mammals, with many similarities in anatomy, physiology, and metabolism of the human gastrointestinal system (Darragh and Hodgkinson 2000; Deglaire and Moughan 2012; Roura et al. 2016). In contrast to rodents, pigs also have the capacity to function as meal eaters allowing for the collection of larger digesta samples (Miller and Ullrey 1987; Moughan and Rowan 1989). Studies have found a good correlation between pigs and humans for apparent and true ileal digestibilities of AAs and nitrogen (Deglaire et al. 2009; Rowan et al. 1994). Compared with true total tract protein digestibility, true ileal AA digestibility is considered to be a superior methodology.

The dual-isotope tracer method has been recently developed as a method to determine true ileal IAA digestibility in humans (Devi et al. 2018). The appearance of labelled AAs in plasma from an intrinsically labelled ingredient protein ($^{2}$H-chickpeas, $^{2}$H-mung beans) is compared with that of a simultaneously ingested and differentially labelled standard protein ($^{13}$C-spirulina). This method has some scientific strengths, such as within-subject quantification of PQ of a novel ingredient to a known ingredient. However, the method requires an isotopically intrinsically labelled food to be developed (labelling chickpeas or mung beans by growing them in a controlled environment with the provision of $^{2}H_2O$). Subsequently, measurement of the appearance of labeled AAs in the plasma pool following administration of small meals is performed in a clinical unit. The method has not been validated against any existing methods. Furthermore, the method is clearly very expensive to apply, cannot be routinely conducted on all food ingredients, and requires specific expertise to create isotopically labelled foods and conduct isotope dilution studies.

Animal nutritionists rely on primary protein ingredients that can represent more than 80% of the ileal digestible limiting AA when formulating diets based on ileal digestibility. However, human foods recorded within the USDA nutrient profile database currently contain 247 000 foods (USDA 2019). The sheer number of foods, the difficulty of determining individual ileal digestible AA coefficients, and the regulatory aspects required in human foods create significant and practical challenges for implementing ileal digestibility coefficients to estimate PQ for human foods.

## Applying digestibility to measures of PQ

### The PDCAAS

PDCAAS was the first method to be adopted that used an indirect measure of IAA bioavailability as total tract digestibility (FAO/WHO 1991), and was presented as an alternative to the rat PER. PDCAAS is calculated as the product of the lowest IAA score and the true total tract protein digestibility (FAO/WHO 1991). The AA scores are calculated as the ratio between the IAA in the food (milligrams per gram of protein) and the IAA requirements of a reference population. The lowest AA score is considered the first-limiting AA. Detailed information on PDCAAS calculations have been extensively discussed (FAO/WHO 1991; Marinangeli and House 2017; Supplementary Fig. S2[1]). In the United States of America, PDCAAS is the official method for determining the PQ of foods consumed by humans ≥ 4 years old.

The PDCAAS for a food can be readily calculated from data in the literature on AA content and true total tract protein digestibility of the ingredient. Since its inception, several criticisms and caveats regarding PDCAAS have been discussed (Sarwar 1997; Schaafsma 2000, 2005). As described previously (Fig. 1), the use of total tract protein digestibility can overestimate AA bioavailability of protein from ingredients with poor ileal digestibility (Rutherfurd et al. 2015). Furthermore, PDCAAS values are truncated to 1.0, reflecting the notion that AAs supplied above requirements do not have additional physiological value and will be catabolised. This truncation can underestimate the high PQ value of some ingredients (e.g., eggs and cow's milk), impedes the ability to compare the PQ among ingredients, and does not allow low-quality ingredients to be compensated for when consuming a combination of complementary proteins (Sarwar 1997).

For PDCAAS, the rat is considered an acceptable model for determining true total tract protein digestibility (FAO/WHO 1991). Behaviourally, rats possess several characteristics, such as coprophagy, that may alter true digestion of a protein. Recommendations are provided to assist in the prevention of coprophagy (e.g., wire bottom cages). Otherwise, overestimation of protein digestibility may be expected (Fuller and Tome 2005).

Finally, the calculation of the PDCAAS is dependent on the reference pattern used to calculate IAA scores. Thus, use of an IAA reference pattern for a growing population, such as pre-school children (as per the FAO/WHO 1991 report) can underestimate PQ of a food consumed by adult populations that require protein for maintenance and not growth (Table 3). Reference IAA patterns for growing populations are often used for determining the PQ of a food for labelling because it provides a more conservative estimate of PQ.

### Digestible indispensable AA score

DIAAS was developed to address some of the methodological limitations identified with PDCAAS. Similarly, DIAAS requires measurement of the IAA content of the ingredient. Unlike PDCAAS, however, DIAAS uses true ileal digestibility of individual IAA. The total IAA in the ingredient is multiplied by the ileal AA digestibility coefficient to get the total ileal digestible IAA. The supply of each AA is compared with the AA requirements of the reference population (by age; FAO 2013), and the lowest value, expressed as percentage, is DIAAS within that particular protein ingredient. Compared with PDCAAS, there is no truncation for DIAAS values, and some protein ingredients can have values greater than 100. The FAO (2013) has recommended that PQ be

**Table 3.** IAA requirements (mg/g CP) for humans and rats at different life stages.

| | Human, years | | | | | | Rat | |
|---|---|---|---|---|---|---|---|---|
| IAA | 0.5 | 1–2 | 3–10 | 11–14 | 15–18 | >18 | Growth | Maintenance |
| Aromatic AA | 52 | 46 | 41 | 41 | 40 | 38 | 68 | 38 |
| Histidine | 20 | 18 | 16 | 16 | 16 | 15 | 18.7 | 16 |
| Ile | 32 | 31 | 30 | 30 | 30 | 30 | 41.3 | 62 |
| Leu | 66 | 63 | 61 | 61 | 60 | 59 | 71.3 | 36 |
| Lys | 57 | 52 | 48 | 48 | 47 | 45 | 61.3 | 22 |
| Sulfur AA | 27 | 25 | 23 | 23 | 23 | 22 | 65.3 | 46 |
| Thr | 31 | 27 | 25 | 25 | 24 | 23 | 41.3 | 36 |
| Trp | 8.5 | 7 | 6.6 | 6.6 | 6.3 | 6 | 13.3 | 10 |
| Val | 43 | 41 | 40 | 40 | 40 | 39 | 49.3 | 46 |

**Note:** Adapted from FAO (2013) and NRC (1995). AA, amino acid; IAA, indispensable AA.



evaluated using the DIAAS method within regulatory frameworks, but DIAAS has yet to be adopted by any regulatory agency. The dearth of human-determined ileal digestibility coefficients published in literature might hamper widespread use of DIAAS; however, there are a growing number of animal-determined ileal digestibility coefficients that could contribute to the adoption of DIAAS within a regulatory framework (Hodgkinson et al. 2018; Abelilla et al. 2018; Rutherfurd et al. 2015). Similar to PDCAAS, the reference pattern used to calculate DIAAS will affect the calculated PQ of the food. Therefore, the correct reference pattern should be used to avoid inaccurate estimation of PQ for different life stages (Rutherfurd et al. 2015).

## In vitro methods for determining the digestibility of foods

Both PDCAAS and DIAAS analyses require digestibility coefficients of the protein or IAA for the test food. Given the plethora of foods and food-based ingredients available, as well the ongoing development of new food technologies, the availability of digestibility coefficients is often the rate-limiting step to using either the PDCAAS or DIAAS method. Furthermore, in vivo analysis of digestibility can be resource intensive, time consuming, and costly, which can hamper the generation of PQ scores for human food. If demonstrated to be an accurate representation of in vivo digestibility, robust in vitro digestibility methods have been developed that may potentially enable the calculation of PDCAAS and DIAAS. In vitro assays are less expensive to conduct, not subjected to ethical constraints, and can reduce variability related to biological differences among subjects (Fernández-Garciá et al. 2009). Longland (1991) proposed five conditions for reliable in vitro digestibility models: (1) sequential use of digestive enzymes at physiological concentrations, (2) an appropriate environment for enzymes to act (e.g., temperature, pH, and presence of cofactors), (3) appropriate mixing of digesta and enzymes, (4) physiological transit time, and (5) removal of products of digestion. The following sections will discuss dynamic and static in vitro digestibility methods currently used as a proxy for in vivo models.

### Artificial gut systems

Artificial gut systems are dynamic models that closely mimic the digestion, absorption, and passage of a food through the gastrointestinal tract. The TIM (Triskelion, Netherlands) is an artificial gut system that can be further classified into different TIM systems (e.g., TIM-1). The TIM-1 is described as a computer-controlled dynamic model of the gastrointestinal tract (Minekus et al. 1995) that consists of four compartments that simulate the stomach, duodenum, jejunum, and ileum of a monogastric animal. Temperature, digesta passage rate, and pH of the gastric and duodenal compartments can be controlled, and digestive enzymes (e.g., amylase, lipase, pepsin) and bile can be added to simulate digestion of macronutrients. Moreover, hollow fibre-dialysis membranes attached to the jejunum and ileum compartments permit products of digestion to be removed and avoids enzyme inhibition and maintains continuous digestion. In addition to the TIM system, other artificial gut systems are also available (Dupont et al. 2019).

Across five diets, Minekus (1998) demonstrated high coefficients of determination between in vivo and TIM-1 digestibility of dry matter ($r^2$ = 0.82) and protein ($r^2$ = 0.95). Havenaar and colleagues (2016) used the tiny-TIM, to simulate similar gastric conditions as demonstrated in humans, and were successful at calculating DIAAS values using this in vitro digestion model from in vitro individual AA digestibility. However, the dialysis membranes may not account for active transport. Moreover, control of pH and peristaltic movements, and immediate feedback from anti-nutritional components on digestion are difficult to mimic in vitro. Before artificial gut systems are used as replacement for in vivo models, users are required to validate dynamic gut systems to ensure digestibility AA coefficients reflect the PQ of a food when consumed by humans.

### In vitro methodologies: static methods

Alternative to dynamic models, static digestion models have also been utilized for many decades to estimate protein digestibility coefficients for human and animal foods (Bodwell et al. 1980; Boisen and Eggum 1991; Butts et al. 2012; Hur et al. 2011; Moughan 1999; Savoie 1994). The pH-drop (Hsu et al. 1977) and pH-stat (Pedersen and Eggum 1983) approaches are methods that use regression equations to calculate changes in pH or the consumption of alkali, respectively, when the test protein is subject to a limited number of digestive enzymes. Alternatively, digestion methods such as pepsin-pancreatin or two-step digestion methodologies place an emphasis on digestion parameters relevant to simulating both gastric and small intestinal digestion (Moughan 1999). The in vitro digestibility is typically calculated as the remaining soluble nitrogen post digestion relative to the initial total nitrogen within the foodstuff, which may include additional filtration, centrifugation, or size exclusion prior to analysis (Dumas combustion, Kjeldahl, spectrophotometry, or chromatography).

Static digestibility methods do not simulate all parameters of digestion. Nevertheless, relatively strong correlations in the pH-drop method and between apparent ($r$ = 0.90, $n$ = 23) and total tract protein digestibility ($r$ = 0.93, $n$ = 18) have been demonstrated (Hsu et al. 1977; Queiroz Mendes et al. 2016). Similarly, the pH-stat method is correlated with true total tract protein digestibility ($r$ = 0.96, $n$ = 30), with high reproducibility across laboratories (McDonough et al. 1990; Pedersen and Eggum 1983). The two-step methodologies have produced correlations with apparent total tract protein digestibility of few ($r$ = 0.98, $n$ = 7) combinations of proteins (Furuya et al. 1979), as well as apparent ileal protein digestibility of plant and animal proteins ($r$ = 0.96, $n$ = 48), or several combinations ($r$ = 0.75, $n$ = 15) of proteins (Boisen and Fernandez 1997). Standardized approaches of two-step digestion methods have been suggested (Hollebeeck et al. 2013; Minekus 2015; Minekus et al. 2014), but have yet to be validated with in vivo digestibility data within the context of PDCAAS.

Static digestion models have been used to estimate PDCAAS (Boye et al. 2012). Modified pH-stat ($r = 0.96$), and a two-step digestion method ($r = 0.98$), relative to some ($n = 7$) plant-based proteins and casein have demonstrated strong correlations with PDCAAS (Rozan et al. 1997). Likewise, using digestibility values acquired by the pH-drop, in vitro PDCAAS showed a strong correlation ($r > 87$) with in vivo PDCAAS in a variety of cooked pulses (Nosworthy et al. 2018*a*, 2018*b*, 2017; Nosworthy and House 2017; Tavano et al. 2016), although static in vitro methods seem accurate and repeatable to evaluate total tract protein digestibility as a surrogate to in vivo total tract protein digestibility for the calculation of PDCAAS. Depending on the category of food, in vitro methods may require further standardization, and validation when the generated digestibility coefficients are used to calculate PDCAAS. To our knowledge, static in vitro digestibility methods have not been successful in determining accurate individual AA digestibility coefficients. Thus, true fecal total tract nitrogen digestibility is currently the only validated in vitro method used for PDCAAS and no in vitro method has been validated for individual AA digestibility for use to measure DIAAS.

### Isotopic methods for the determining whole-body use of AAs

The indicator AA is an IAA with the labelled carbon or nitrogen irreversibly lost as $CO_2$ or urea, respectively, during the first steps of oxidation (Levesque et al. 2010). Lysine (Ball and Bayley 1984), threonine (Soliman and King 1969), [$^{14}$C-methyl]-methionine (Brookes et al. 1972), and leucine (Hsu et al. 2006; Kurpad et al. 1998) have been used as indicator AAs. However, 1-$^{14}$C- or 1-$^{13}$C-phenylalanine, in the presence of excess tyrosine, is the preferred indicator AA because of the small free phenylalanine pool that reduces the time required to reach steady state and the amount of isotope used. The indicator AA when administered orally or intravenously provides a steady supply of the AA, but when the indicator AA is provided orally, digestion and absorption of the indicator AA aligns with that of dietary protein. Thus, oral and frequent (30 min) delivery of the isotope is the preferred method of administration (Levesque et al. 2010). Similarly, $^{15}$N-labelled foods can be ingested and ileal, fecal, plasma, and urine nitrogen enrichment can be measured to determine the NPPU. In addition to measuring the metabolic fate of dietary nitrogen and digestibility (Marchini et al. 1993), it allows for shorter term experiments than those required for NPU determinations. This method has the same limitations as the dual-isotope tracer, as it requires an intrinsically labelled protein to be developed first, and clearly not appropriate for routine application in all human foods.

The adaptation to each of the test diets during the IAAO and NPPU technique are relatively short (≤2 days), but additional time may be required when fibrous ingredients are used due to greater time required for adaption by the gut (FAO 2014). For IAAO, at least four graded levels of the test protein are added to a highly digestible protein-free diet with marginal excess of all other nutrients. Across the levels of inclusion, intake of the limiting AA should be below its requirement and intake of the indicator AA must be maintained relative to control for its partitioning between protein synthesis and catabolic pathways. Within each IAAO study, and during the steady fed state, the subjects consume constant doses or receive continuous infusion of the isotopically labelled indicator AA. The rate of indicator AA catabolism during steady state decreases as the intake of the limiting AA increases with higher inclusion of the test protein towards protein synthesis (Moehn et al. 2005). The test protein slope is compared with that obtained when the test protein is replaced with equal protein portions of a reference protein (i.e., casein). The ratio of the slopes expressed as a percentage is defined as the PQ ranking.

Across a wide range of lysine intakes in humans, Elango et al. (2009) found that protein intake for 2 days of adaptation was sufficient to estimate individual AA requirements compared with 10 days. Given the minimal invasiveness of the technique, and the short period of time required for adaptation to diets, the IAAO technique can be used for reliable PQ assessment of ingredients for subjects at different life stages, including vulnerable populations such as pregnant and lactating women and the elderly (Elango et al. 2008). The experimental requirements for IAAO technique are similar to that of the PER where proteins are compared with a selected reference protein, such as casein. Moreover, similar to PER, the ranking of proteins is not linear across different foods and, as such, comparison of the different PQ values should be done carefully. Finally, in contrast to digestibility methods, the IAAO method can detect reduced bioavailability of AA and, therefore, PQ of heat-treated ingredients.

## Conclusions

There are several methods for determining PQ for human food, with varying advantages and disadvantages (Supplementary Table S1[1]). The PER can be used for determining PQ during stages of rapid growth, such as in infants and toddlers. However, PER values are not additive and animal models typically used to determine the PER of foods have IAA requirements that differ from those of humans. Thus, the PER of some ingredients and foods can be underestimated in the context of human diets. Digestibility-based methods for PQ have been accepted as a replacement for PER. PDCAAS is a digestibility-based method that incorporates true total tract protein digestibility and the IAA requirements of humans. However, the use of true total tract protein digestibility is confounded by the absorption of nitrogen and microbial activity in the large intestine and discrepancies between AA and protein digestibility. The DIAAS methodology is a superior method for the assessment of PQ compared with PDCAAS. Attaining true ileal digestibility coefficients for humans is inherently difficult, although the pig model has been deemed a suitable model. Nevertheless, the time cost, invasiveness, and ethical constraints required for determining ileal digestibility coefficients remains an issue. As an alternative, in vitro dynamic systems have been developed for true total and ileal nitrogen digestibility, but further validation of the methodology as an alternative for determining ileal digestibility of individual AAs is warranted. Static methods could be used as a proxy for total tract protein digestibility and could be sufficient for calculating the PDCAAS of foods. The IAAO technique is a powerful and versatile option when determining PQ and bioavailability of AAs. However, similar to PER, values determined with IAAO technique are not additive and the method requires more complicated materials and equipment, resulting in time and expense. However, the bioavailability obtained using the IAAO method accounts for digestion, absorption, and cellular metabolism and is less likely to underestimate AA availability than digestibility methods.

As discussed in this review, each PQ method demonstrates strengths and weaknesses. From a scientific perspective, DIAAS is considered to be the most accurate representation of PQ, but may not address the disappearance of AAs that may be otherwise unavailable for protein synthesis. However, depending of the application of PQ, other methods could be sufficient. Thus, without knowing the application, it remains difficult to choose one over another. To do so, an understanding of the context under which the method will be utilized as well as available resources are required. Understanding of the advantages and disadvantages of each method will assist with selecting a measure of PQ that can best address the needs of a study, a population, or regulation.

### Conflict of interest statement

C.P.F.M. works for Pulse Canada and is a former employee of Kellogg Canada. W.D.M. works for Trouw Nutrition, Spain. C.C.F., A.F., J.D.H., R.E., D.A.C., E.K., M.R., and A.K.S. have no conflicts of interest.

## Acknowledgements

This review article was undertaken thanks in part to funding from the Canada First in Research Excellence Fund. All authors contributed to the content of this paper and are listed from greatest to least input, with the exception of the corresponding author.

## References

Abelilla, J.J., Liu, Y., and Stein, H.H. 2018. Digestible indispensable amino acid score (DIAAS) and protein digestibility corrected amino acid score (PDCAAS) in oat protein concentrate measured in 20- to 30-kilogram pigs. J. Sci. Food. Agric. **98**: 410–414. doi:10.1002/jsfa.8457. PMID:28573795.

Adeola, A. 2001. Digestion and balance techniques in pigs. *In* Swine nutrition. *Edited by* A.J. Lewis and L.L. Southern. CRC Press, Boca Raton, Fla. pp. 903–916.

Ball, R.O., and Bayley, H.S. 1984. Tryptophan requirement of the 2.5-kg piglet determined by the oxidation of an indicator amino acid. J. Nutr. **114**(10): 1741–1746. doi:10.1093/jn/114.10.1741. PMID:6434707.

Batal, A.B., and Parsons, C.M. 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poult. Sci. **81**(3): 400–407. doi:10.1093/ps/81.3.400. PMID:11902418.

Batterham, E.S. 1992. Availability and utilization of amino acids for growing pigs. Nutr. Res. Rev. **5**(1): 1–18. doi:10.1079/NRR19920004. PMID:19094310.

Beach, E.F., Bernstein, S.S., Hoffman, O.D., Teague, D.M., and Macy, I.G. 1941. Distribution of nitrogen and protein amino acids in human and in cow's milk. J. Biol. Chem. **139**(1): 57–63.

Bodwell, C.E., Satterlee, L.D., and Hackler, L.R. 1980. Protein digestibility of the same protein preparations by human and rat assays and by in vitro enzymic digestion methods. Am. J. Clin. Nutr. **33**(3): 677–686. doi:10.1093/ajcn/33.3.677. PMID:6986763.

Boisen, S., and Eggum, B.O. 1991. Critical evaluation of in vitro methods for estimating digestibility in simple-stomach animals. Nutr. Res. Rev. **4**(1): 141–162. doi:10.1079/NRR19910012. PMID:19094329.

Boisen, S., and Fernández, J.A. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed Sci. Technol. **68**(3): 277–286. doi:10.1016/S0377-8401(97)00058-8.

Boling-Frankenbach, S.D., Peter, C.M., Douglas, M.W., Snow, J.L., Parsons, C.M., and Baker, D.H. 2001. Efficacy of phytase for increasing protein efficiency ratio values of feed ingredients. Poult. Sci. **80**(11): 1578–84. doi:10.1093/ps/80.11.1578. PMID:11732674.

Boye, J., Wijesinha-Bettoni, R., and Burlingame, B. 2012. Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. Br. J. Nutr. **108**(Suppl. 2): S183–S211. doi:10.1017/S0007114512002309. PMID:23107529.

Brookes, I.M., Owens, F.N., and Garrigus, U.S. 1972. Influence of amino acid level in the diet upon amino acid oxidation by the rat. J. Nutr. **102**(1): 27–35. doi:10.1093/jn/102.1.27. PMID:5007115.

Buamah, T.F., and Singsen, E.P. 1975. Studies on the protein efficiency ratio method for the evaluation of poultry feed supplements. Modifications associated with choice of dietary protein level for assay. J Nutr. **105**(6): 688–700. doi:10.1093/jn/105.6.688. PMID:1170286.

Butts, C.A., Monro, J.A., and Moughan, P.J. 2012. In vitro determination of dietary protein and amino acid digestibility for humans. Br. J. Nutr. **108**(S2): S282–S287. doi:10.1017/S0007114512002310. PMID:23107539.

Chapman, D.G., Castillo, R., and Campbell, J.A. 1959. Evaluation of protein in foods. I. A method for the determination of protein efficiency ratios. Can. J. Biochem. Physiol. **37**(5): 679–686. doi:10.1139/o59-074. PMID:13651969.

Columbus, D., and de Lange, C.F. 2012. Evidence for validity of ileal digestibility coefficients in monogastrics. Br. J. Nutr. **108**(Suppl. 2): S264–S272. doi:10.1017/S0007114512002334. PMID:23107537.

Columbus, D.A., Lapierre, H., Htoo, J.K., and de Lange, C.F. 2014. Nonprotein nitrogen is absorbed from the large intestine and increases nitrogen balance in growing pigs fed a valine-limiting diet. J. Nutr. **144**(5): 614–620. doi:10.3945/jn.113.187070. PMID:24647394.

Darragh, A.J., and Hodgkinson, S.M. 2000. Quantifying the digestibility of dietary protein. J. Nutr. **130**(7): 1850S–1856S. doi:10.1093/jn/130.7.1850S. PMID:10867062.

Darragh, A.J., Cranwell, P.D., and Moughan, P.J. 1994. Absorption of lysine and methionine from the proximal colon of the piglet. Br. J. Nutr. **71**(5): 739–752. doi:10.1079/BJN19940181. PMID:8054329.

de Lange, C.F., Sauer, W.C., Mosenthin, R., and Souffrant, W.B. 1989. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. **67**(3): 746–54. doi:10.2527/jas1989.673746x. PMID:2722704.

Deglaire, A., and Moughan, P.J. 2012. Animal models for determining amino acid digestibility in humans - a review. Br. J. Nutr. **108**(Suppl. 2): S273–S281. doi:10.1017/S0007114512002346. PMID:23107538.

Deglaire, A., Bos, C., Tomé, D., and Moughan, P.J. 2009. Ileal digestibility of dietary protein in the growing pig and adult human. Br. J. Nutr. **102**(12): 1752–1759. doi:10.1017/S0007114509991267. PMID:19706206.

Devi, S., Varkey, A., Sheshshayee, M.S., Preston, T., and Kurpad, A.V. 2018. Measurement of protein digestibility in humans by a dual-tracer method. The American Journal of Clinical Nutrition **107**(6): 984–991. doi:10.1093/ajcn/nqy062. PMID:29771297.

Dierick, N.A., Vervaeke, I.J., Decuypere, J.A., and Henderickx, H.K. 1986. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs. II. Studies in vivo. Livest. Prod. Sci. **14**(2): 177–193. doi:10.1016/0301-6226(86)90006-0.

Dowsett, J., Gibney, M., and Kennedy, N. 1990. Bacterial fermentation occurs in the terminal ileum of ileostomates. Proc. Nutr. Soc. **49**: 110.A.

Dupont, D., Alric, M., Blanquet-Diot, S., Bornhorst, G., Cueva, C., Deglaire, A., et al. 2019. Can dynamic in vitro digestion systems mimic the physiological reality? Crit. Rev. Food Sci. Nutr. **59**(10): 1546–1562. doi:10.1080/10408398.2017.1421900. PMID:29359955.

Elango, R., Ball, R.O., and Pencharz, P.B. 2008. Indicator amino acid oxidation: concept and application. J. Nutr. **138**(2): 243–246. doi:10.1093/jn/138.2.243. PMID:18203885.

Elango, R., Humayun, M.A., Ball, R.O., and Pencharz, P.B. 2009. Indicator amino acid oxidation is not affected by period of adaptation to a wide range of lysine intake in healthy young men. J. Nutr. **139**(6): 1082–1087. doi:10.3945/jn.108.101147. PMID:19369367.

Elango, R., Ball, R.O., and Pencharz, P.B. 2012. Recent advances in determining protein and amino acid requirements in humans. Br. J. Nutr. **108**(Suppl 2): S22–S30. doi:10.1017/s0007114512002504. PMID:23107531.

Fernandez, S., and Parsons, C.M. 1996. Bioavailability of digestible lysine in heat-damaged soybean meal for chick growth. Poult. Sci. **75**(2): 224–231. doi:10.3382/ps.0750224. PMID:8833375.

Fernández-García, E., Carvajal-Lérida, I., and Pérez-Gálvez, A. 2009. In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. Nutr. Res. **29**(11): 751–760. doi:10.1016/j.nutres.2009.09.016. PMID:19932863.

Food and Agriculture Organization (FAO). 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. FAO Food Nutr. Pap. **92**:1–66. PMID:26369006.

Food and Agriculture Organization (FAO). 2014. The state of food and agriculture: innovation in family farming. FAO, Rome, Italy.

Food and Agriculture Organization/World Health Organization (FAO/WHO). 1991. Protein quality evalutation. FAO Food Nutr. Pap. **51**: 1–66. PMID:1817076.

Food and Drug Administration (FDA). 2018. 21CFR101.9. U.S Food and Drug Administration, Silver springs, Md.

Fuller, M. 2012. Determination of protein and amino acid digestibility in foods including implications of gut microbial amino acid synthesis. Br. J. Nutr. **108**(S2): S238–S246. doi:10.1017/S0007114512002279. PMID:23107534.

Fuller, M.F., and Reeds, P.J. 1998. Nitrogen cycling in the gut. Annu. Rev. Nutr. **18**(1): 385–411. doi:10.1146/annurev.nutr.18.1.385. PMID:9706230.

Fuller, M.F., and Tomé, D. 2005. In vivo determination of amino acid bioavailability in humans and model animals. J. AOAC. Int. **88**(3): 923–934. doi:10.1093/jaoac/88.3.923. PMID:16001870.

Fuller, M.F., McWilliam, R., Wang, T.C., and Giles, L.R. 1989. The optimum dietary amino acid pattern for growing pigs: 2. Requirements for maintenance and for tissue protein accretion. Br. J. Nutr. **62**(2): 255–267. doi:10.1079/bjn19890028. PMID:2819011.

Furuya, S., Sakamoto, K., and Takahashi, S. 1979. A new in vitro method for the estimation of digestibility using the intestinal fluid of the pig. Br. J. Nutr. **41**(3): 511–520. doi:10.1079/BJN19790066. PMID:380637.

González-Vega, J.C., Kim, B.G., Htoo, J.K., Lemme, A., and Stein, H.H. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. J. Anim. Sci. **89**(11): 3617–3625. doi:10.2527/jas.2010-3465. PMID:21742940.

Government of Canada. 1981. Method FO-1: Determination of Protein Rating. Health Canada, Ottawa, ON, Canada.

Havenaar, R., Maathuis, A., de Jong, A., Mancinelli, D., Berger, A., and Bellmann, S. 2016. Herring roe protein has a high digestible indispensable amino acid score (DIAAS) using a dynamic in vitro gastrointestinal model. Nutr. Res. **36**(8): 798–807. doi:10.1016/j.nutres.2016.05.004. PMID:27440534.

Health Canada. 1981. Chemical methods - Compendium of methods for chemical analysis of foods: Method FO-1. Available from https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/chemical-compendium-analysis-foods.html.

Hendriks, W.H., van Baal, J., and Bosch, G. 2012. Ileal and faecal protein digestibility measurement in humans and other non-ruminants - a comparative species view. Br. J. Nutr. **108**(S2): S247–S257. doi:10.1017/S0007114512002395. PMID:23107535.

Hodgkinson, S.M., Montoya, C.A., Scholten, P.T., Rutherfurd, S.M., and Moughan, P.J. 2018. Cooking conditions affect the true ileal digestible amino acid content and digestible indispensable amino acid score (DIAAS) of bovine meat as determined in pigs. J. Nutr. **148**(10): 1564–1569. doi:10.1093/jn/nxy153. PMID:30204886.

Hoffer, L.J. 2016. Human protein and amino acid requirements. J. Parenter. Enteral. Nutr. **40**(4): 460–474. doi:10.1177/0148607115624084. PMID:26796095.

Hollebeeck, S., Borlon, F., Schneider, Y.J., Larondelle, Y., and Rogez, H. 2013. Development of a standardised human in vitro digestion protocol based on macronutrient digestion using response surface methodology. Food Chem. **138**(2–3): 1936–1944. doi:10.1016/j.foodchem.2012.11.041. PMID:23411328.

Hsu, H.W., Vavak, D.L., Satterlee, L.D., and Miller, G.A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. **42**(5): 1269–1273. doi:10.1111/j.1365-2621.1977.tb14476.x.

Hsu, J.W., Kriengsinyos, W., Wykes, L.J., Rafii, M., Goonewardene, L.A., Ball, R.O., et al. 2006. Leucine is not a good choice as an indicator amino acid for determining amino acid requirements in men. J. Nutr. **136**(4): 958–964. doi:10.1093/jn/136.4.958. PMID:16549457.

Humayun, M.A., Elango, R., Moehn, S., Ball, R.O., and Pencharz, P.B. 2007. Application of the indicator amino acid oxidation technique for the determination of metabolic availability of sulfur amino acids from casein versus soy protein isolate in adult men. J. Nutr. **137**(8): 1874–1879. doi:10.1093/jn/137.8.1874. PMID:17634258.

Hur, S.J., Lim, B.O., Decker, E.A., and McClements, D.J. 2011. In vitro human digestion models for food applications. Food Chem. **125**(1): 1–12. doi:10.1016/j.foodchem.2010.08.036.

Jensen, B.B., and Jørgensen, H. 1994. Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Appl. Environ. Microbiol. **60**(6): 1897–1904. doi:10.1128/AEM.60.6.1897-1904.1994. PMID:8031085.

Just, A., Jørgensen, H., and Fernández, J.A. 1981. The digestive capacity of the caecum-colon and the value of the nitrogen absorbed from the hind gut for protein synthesis in pigs. Br. J. Nutr. **46**(1): 209–219. doi:10.1079/BJN19810022. PMID:6266451.

Just, A., Jørgensen, H., and Fernández, J. 1985. Correlations of protein deposited in growing female pigs to ileal and faecal digestible crude protein and amino acids. Livest. Prod. Sci. **12**(2): 145–159. doi:10.1016/0301-6226(85)90087-9.

Khattab, R.Y., Arntfield, S.D., and Nyachoti, C.M. 2009. Nutritional quality of legume seeds as affected by some physical treatments, Part 1: Protein quality evaluation. Food Sci. Technol. **42**(6): 1107–1112. doi:10.1016/j.lwt.2009.02.008.

Krawielitzki, K., Zebrowska, T., Schadereit, R., Kowalczyk, J., Hennig, U., Wünsche, J., et al. 1990. Determining of nitrogen absorption and nitrogen secretion in different sections of the pig's intestine by digesta exchange between 15N labelled and unlabelled animals. Arch. Anim. Nutr. **40**(1–2): 25–37. doi:10.1080/17450399009428378. PMID:2344273.

Kurpad, A.V., El-Khoury, A.E., Beaumier, L., Srivatsa, A., Kuriyan, R., Raj, T., et al. 1998. An initial assessment, using 24-h [13C] leucine kinetics, of the lysine requirement of healthy adult Indian subjects. Am. J. Clin. Nutr. **67**(1): 58–66. doi:10.1093/ajcn/67.1.58. PMID:9440376.

Levesque, C.L., Moehn, S., Pencharz, P.B., and Ball, R.O. 2010. Review of advances in metabolic bioavailability of amino acids. Livest. Sci. **133**(1–3): 4–9. doi:10.1016/j.livsci.2010.06.013.

Libao-Mercado, A.J., Zhu, C.L., Cant, J.P., Lapierre, H., Thibault, J.N., Seve, B., et al. 2009. Dietary and endogenous amino acids are the main contributors to microbial protein in the upper gut of normally nourished pigs. J. Nutr. **139**(6): 1088–1094. doi:10.3945/jn.108.103267. PMID:19403708.

Longland, A.C. 1991. Digestive enzyme activities in pigs and poultry. *In* In vitro digestion for pigs and poultry. *Edited by* M.F. Fuller. CAB International, Wallingford, UK. pp. 3–18.

Low, A.G. 1980. Nutrient absorption in pigs. J. Sci. Food Agric. **31**(11): 1087–1130. doi:10.1002/jsfa.2740311102. PMID:7017272.

Low, A.G., Partridge, I.G., Keal, H.D., and Jones, A.R. 1982. A comparison of methods in vitro and in vivo measuring amino acid digestibility in foodstuffs as predictors of pig growth and carcass composition. Anim. Prod. **34**: 413.

Mansilla, W.D., Columbus, D.A., Htoo, J.K., and de Lange, C.F. 2015. Nitrogen absorbed from the large intestine increases whole-body nitrogen retention in pigs fed a diet deficient in dispensable amino acid nitrogen. J. Nutr. **145**(6): 1163–1169. doi:10.3945/jn.115.212316. PMID:25926407.

Marchini, J.S., Cortiella, J., Hiramatsu, T., Chapman, T.E., and Young, V.R. 1993. Requirements for indispensable amino acids in adult humans: longer-term amino acid kinetic study with support for the adequacy of the Massachusetts Institute of Technology amino acid requirement pattern. The American Journal of Clinical Nutrition, **58**(5): 670–683. doi:10.1093/ajcn/58.5.670. PMID:8237874.

Marinangeli, C.P., and House, J.D. 2017. Potential impact of the digestible indispensable amino acid score as a measure of protein quality on dietary regulations and health. Nut. Rev. **75**(8): 658–667. doi:10.1093/nutrit/nux025. PMID:28969364.

Mason, V.C., and Palmer, R. 1973. The influence of bacterial activity in the alimentary canal of rats on faecal nitrogen excretion. Acta Agric. Scan. **23**(3): 141–150. doi:10.1080/00015127309433211.

Mason, V., Just, A., and Bech-Andersen, S. 1976. Bacterial activity in the hind-gut of pigs: 2. Its influence on the apparent digestibility of nitrogen and amino acids. Z. Tierphysiol. Tierernahr. Futtermittelkd. **36**(1–6): 310–324. doi:10.1111/j.1439-0396.1975.tb01150.x. PMID:961088.

McDonough, F.E., Sarwar, G., Steinke, F.H., Slump, P., Garcia, S., and Boisen, S. 1990. In vitro assay for protein digestibility: interlaboratory study. J. AOAC. Int. **73**: 622–625. doi:10.1093/jaoac/73.4.622. PMID:2211487.

Miller, E.R., and Ullrey, D.E. 1987. The pig as a model for human nutrition. Annu. Rev. Nutr. **7**(1): 361–382. doi:10.1146/annurev.nu.07.070187.002045. PMID:3300739.

Minekus, M. 1998. Development and validation of a dynamic model of the gastrointestinal tract. PhD thesis. University of Utrecht, the Netherlands.

Minekus, M. 2015. The TNO gastro-intestinal model (TIM). *In* The impact of food bioactives on health. *Edited by* K. Verhoeckx. Springer, Cham. pp. 37–46. doi:10.1007/978-3-319-16104-4_5.

Minekus, M., Marteau, P., Havenaar, R., and Huis in 't Veld, J.H.H. 1995. A multicompartmental dynamic computer- controlled model simulating the stomach and small intestine. Altern. Lab. Anim. **23**: 197–209.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., et al. 2014. A standardised static in vitro digestion method suitable for food – an international consensus. Food Funct. **5**(6): 1113–1124. doi:10.1039/C3FO60702J. PMID:24803111.

Moehn, S., Bertolo, R.F., Pencharz, P.B., and Ball, R.O. 2005. Development of the indicator amino acid oxidation technique to determine the availability of amino acids from dietary protein in pigs. J. Nutr. **135**(12): 2866–2870. doi:10.1093/jn/135.12.2866. PMID:16317134.

Moughan, P.J. 1995. Modelling protein metabolism in the pig-critical evaluation of a simple reference model. *In* Modelling growth in the pig. *Edited by* P.J. Moughan, M.W.A. Verstegen, and M.I. Visser-Reyneveld. Wageningen Pers, Wageningen, The Netherlands. pp. 103–112.

Moughan, P.J. 1999. In vitro techniques for the assessment of the nutritive value of feed grains for pigs: a review. Aust. J. Agric. Res. **50**(5): 871–880. doi:10.1071/AR98172.

Moughan, P.J., and Donkoh, A. 1991. Amino acid digestibility in non-ruminants: a review. Rec. Adv. Anim. Nutr. Aust. 172–184.

Moughan, P.J., and Rowan, A. 1989. The pig as a model animal for human nutrition research. N. Z. Nutr. Soc. **14**: 116–123.

Moughan, P.J., Ravindran, V., and Sorbara, J.O. 2014. Dietary protein and amino acids-consideration of the undigestible fraction. Poult. Sci. **93**(9): 2400–2410. doi:10.3382/ps.2013-03861. PMID:25037823.

Murray, S.M., Patil, A.R., Fahey, G.C., Jr., Merchen, N.R., and Hughes, D.M. 1997. Raw and rendered animal by-products as ingredients in dog diets. J. Anim. Sci. **75**(9): 2497–2505. doi:10.2527/1997.7592497x. PMID:9303468.

National Research Council (NRC). 1994. National Research Council (US) Subcommittee on Poultry. Nutrient requirements of poultry: 9th revised edition, 1994. National Academies Press (US), Washington, D.C.

National Research Council (NRC). 1995. National Research Council (US) Subcommittee on Laboratory Animal Nutrition. Nutrient requirements of laboratory animals: 4th revised edition. National Academies Press (US), Washington, D.C.

National Research Council (NRC). 2006. National Research Council (US) Subcommittee on Dogs and Cats. Nutrient requirements of dogs and cats. National Academies Press (US), Washington, D.C.

National Research Council (NRC). 2012. National Research Council (US) Subcommittee on Swine. Nutrient requirements of swine: 11th revised edition. National Academies Press (US), Washington, D.C.

Nosworthy, M.G., and House, J.D. 2017. Factors influencing the quality of dietary proteins: Implications for pulses. Cereal Chem. **94**(1): 49–57. doi:10.1094/CCHEM-04-16-0104-FI.

Nosworthy, M.G., Neufeld, J., Frohlich, P., Young, G., Malcolmson, L., and House, J.D. 2017. Determination of the protein quality of cooked Canadian pulses. Food Sci. Nutr. **5**(4): 896–903. doi:10.1002/fsn3.473. PMID:28748078.

Nosworthy, M.G., Medina, G., Franczyk, A.J., Neufeld, J., Appah, P., Utioh, A., et al. 2018*a*. Effect of processing on the in vitro and in vivo protein quality of red and green lentils (Lens culinaris). Food Chem. **240**: 588–593. doi:10.1016/j.foodchem.2017.07.129. PMID:28946315.

Nosworthy, M.G., Medina, G., Franczyk, A.J., Neufeld, J., Appah, P., Utioh, A., et al. 2018*b*. Effect of processing on the in vitro and in vivo protein quality of beans (Phaseolus vulgaris and Vicia faba). Nutrients, **10**(6): 671. doi:10.3390/nu10060671. PMID:29799474.

Noy, Y., and Sklan, D. 1995. Digestion and absorption in the young chick. Poult. Sci. **74**(2): 366–373. doi:10.3382/ps.0740366. PMID:7724461.

Nyachoti, C.M., de Lange, C.F., McBride, B.W., and Schulze, H. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: a review. Canadian J. Anim. Sci. **77**(1): 149–163. doi:10.4141/A96-044.

Osborne, T.B., Mendel, L.B., and Ferry, E.L. 1919. A method of expressing numerically the growth-promoting value of proteins. J. Biol. Chem. **37**(2): 223–229.

Pedersen, B., and Eggum, B.O. 1983. Prediction of protein digestibility by an in vitro enzymatic pH-stat procedure. Z. Tierphysiol. Tierernahr. Futtermittelkd. **49**(1–5): 265–277. doi:10.1111/j.1439-0396.1983.tb00808.x. PMID:6412478.

Queiroz Mendes, F., De Almeida Oliveira, M.G., Brunoro Costa, N.M., Vieira Pires, C., and Regina Passos, F. 2016. Capability of in vitro digestibility methods to predict in vivo digestibility of vegetal and animal proteins. Arch. Latinoam. Nutr. **66**(1): 5–16.

Rerat, A. 1981. Digestion and absorption of nutrients in the pig. *In* Human nutrition and animal feeding. *Edited by* Bourne, G. H. World. Rev. Nutr. Diet. Vol. 37. pp. 229–287.

Roura, E., Koopmans, S.J., Lallès, J.P., Le Huerou-Luron, I., de Jager, N., Schuurman, T., and Val-Laillet, D. 2016. Critical review evaluating the pig as a model for human nutritional physiology. Nutr. Res. Rev. **29**(1): 60–90. doi:10.1017/S0954422416000020. PMID:27176552.

Ross 2014. Broiler 308: Nutrition specifications. Available from http://tmea.staging.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-308-Broiler-Nutrition-Specs-2014r17-EN.pdf.

Rowan, A.M., Moughan, P.J., Wilson, M.N., Maher, K., and Tasman-Jones, C. 1994. 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. **71**(1): 29–42. doi:10.1079/BJN19940108. PMID:8312239.

Rozan, P., Lamghari, R., Linder, M., Villaume, C., Fanni, J., Parmentier, M., and Méjean, L. 1997. In vivo and in vitro digestibility of soybean, lupine, and rapeseed meal proteins after various technological processes. J. Agric. Food Chem. **45**(5): 1762–1769. doi:10.1021/jf960723v.

Rutherfurd, S.M., Fanning, A.C., Miller, B.J., and Moughan, P.J. 2015. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. J. Nutr. **145**(2): 372–379. doi:10.3945/jn.114.195438. PMID:25644361.

Sakomura, N.K., Silva, E.P., Dorigam, J.C., Gous, R.M., and St-Pierre, N. 2015. Modeling amino acid requirements of poultry. J. Appl. Poult. Res. **24**(2): 267–282. doi:10.3382/japr/pfv024.

Sarwar, G. 1997. The protein digestibility–corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. J. Nutr. **127**(5): 758–764. doi:10.1093/jn/127.5.758. PMID:9164998.

Sauer, W.C., and de Lange, K. 1992. Novel methods for determining protein and amino acid digestibilities in feedstuffs. *In* Modern methods in protein nutrition and metabolism. *Edited by* S. Nissen. Academic Press, San Diego, Calif. pp. 87–120.

Sauer, W.C., and Ozimek, L. 1986. Digestibility of amino acids in swine: results and their practical applications. A review. Livest. Prod. Sci. **15**(4): 367–388. doi:10.1016/0301-6226(86)90076-X.

Savoie, L. 1994. Digestion and absorption of food: usefulness and limitations of in vitro models. Can. J. Physiol. Pharmacol. **72**(4): 407–414. doi:10.1139/y94-060. PMID:7922873.

Schaafsma, G. 2000. The protein digestibility–corrected amino acid score. J. Nutr. **130**(7): 1865S–1867S. doi:10.1093/jn/130.7.1865S. PMID:10867064.

Schaafsma, G. 2005. The protein digestibility-corrected amino acid score (PDCAAS)–a concept for describing protein quality in foods and food ingredients: a critical review. J. AOAC. Int. **88**(3): 988–994. doi:10.1093/jaoac/88.3.988. PMID:16001875.

Schulze, H., Van Leeuwen, P., Verstegen, M., Huisman, J., Souffrant, W., and Ahrens, F. 1994. Effect of level of dietary neutral detergent fiber on ileal apparent digestibility and ileal nitrogen losses in pigs. J. Anim. Sci. **72**(9): 2362–2368. doi:10.2527/1994.7292362x. PMID:8002455.

Soliman, A.G.M., and King, K.W. 1969. Metabolic derangements in response of rats to ingestion of imbalanced amino acid mixtures. J. Nutr. **98**(3): 255–270. doi:10.1093/jn/98.3.255. PMID:5796413.

Souffrant, W. 2001. Effect of dietary fibre on ileal digestibility and endogenous nitrogen losses in the pig. Anim. Feed Sci. Technol. **90**(1–2): 93–102. doi:10.1016/S0377-8401(01)00199-7.

Stein, H.H., Seve, B., Fuller, M.F., Moughan, P.J., and de Lange, C.F. 2007. Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J. Anim. Sci. **85**(1): 172–180. doi:10.2527/jas.2005-742. PMID:17179553.

Tanksley, T., and Knabe, D. 1993. Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. *In* Recent advances in animal nutrition ed. 2. *Edited by* W. Haresign, and D.J.A. Cole. Butterworths, London. pp. 75–95.

Tavano, O.L., Neves, V.A., and da Silva, S.I., Júnior 2016. In vitro versus in vivo protein digestibility techniques for calculating PDCAAS (protein digestibility-corrected amino acid score) applied to chickpea fractions. Food Res. Intl. **89**: 756–763. doi:10.1016/j.foodres.2016.10.005.

U.S. Department of Agriculture (USDA). 2019. FoodData Central. Available from http://fdc.nal.usda.gov.

van der Wielen, N., Moughan, P.J., and Mensink, M. 2017. Amino acid absorption in the large intestine of humans and porcine models. J. Nutr. **147**(8): 1493–1498. doi:10.3945/jn.117.248187. PMID:28615378.

Webb, K.E., Jr. 1990. Intestinal absorption of protein hydrolysis products: a review. J. Anim. Sci. **68**(9): 3011–3022. doi:10.2527/1990.6893011x. PMID:2211428.

Williamson, M.B. 1944. The amino acid composition of human milk proteins. J. Biol. Chem. **156**: 47–52.

Zebrowska, T. 1975. Apparent digestibility of nitrogen and individual amino acids in the large intestine of pigs. Rocz. Nauk. Roln. **97B**: 117–123.

Zebrowska, T., and Buraczewski, S. 1998. Methods for determination of amino acids bioavailability of pigs-review. Asian-Australas. J. Anim. Sci. **11**(5): 620–633. doi:10.5713/ajas.1998.620.