DIGESTIBLE INDISPENSABLE AMINO ACID SCORES FOR FOOD PROTEINS

BY

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DISSERTATION

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ABSTRACT: Three experiments were conducted to determine protein quality and to evaluate digestible indispensable amino acid scoring (DIAAS) methodology. Experiment 1 was conducted to compare protein digestibility corrected amino acid scores (PDCAAS) and DIAAS for various plant and animal proteins. Values for standardized total tract digestibility (STTD) of crude protein (CP) and standardized ileal digestibility (SID) of amino acids were calculated for whey protein isolate (WPI), whey protein concentrate (WPC), milk protein concentrate (MPC), skim milk powder (SMP), pea protein concentrate (PPC), soy protein isolate (SPI), soy flour, and whole grain wheat. The PDCAAS-like values were calculated using the STTD of CP to estimate amino acid digestibility and values for DIAAS were calculated from values for SID of amino acids. Results indicated that values for SID of most indispensable amino acids in WPI, WPC, and MPC were greater ($P < 0.05$) than for SMP, PPC, SPI, soy flour, and wheat. If the same scoring pattern for children between 6 and 36 months was used to calculate PDCAAS-like values and DIAAS, PDCAAS-like values were greater ($P < 0.05$) than DIAAS values for SMP, PPC, SPI, soy flour, and wheat indicating that PDCAAS-like values estimated in pigs may overestimate the quality of these proteins. Experiment 2 was conducted to determine the DIAAS values for pork loin and to evaluate the effect of roasting, frying, or grilling of pork loin on protein quality. The DIAAS were calculated based on ileal digestibility of amino acids in pigs for raw pork loin, roasted pork loin, grilled pork loin, fried pork loin, and casein. Six ileal-cannulated barrows were allotted to a 6 × 6 Latin square design with 6 diets and 6 periods during which ileal effluent samples were collected to determine amino acid digestibility. A N-free diet was formulated to determine basal endogenous losses of amino acids and crude protein (CP) and to enable the calculation of standardized ileal digestibility (SID) of amino acids. The remaining diets were formulated with each test ingredient as the sole source of amino acids. Using determined values

for SID of amino acids for each ingredient and established reference protein patterns, DIAAS were calculated. For children from birth to 6 m, fried pork loin had the greatest ($P < 0.05$) DIAAS followed by grilled pork loin, roasted pork loin, raw pork loin, and casein. For children from 6 m to 3 y, DIAAS were greatest ($P < 0.05$) for grilled and fried pork loin and least ($P < 0.05$) for raw pork loin and the DIAAS of roasted pork loin was greater ($P < 0.05$) than that of casein. For DIAAS calculated for children older than 3 y, there were no differences in the DIAAS among grilled pork loin, fried pork loin, and casein, but these 3 ingredients had greater ($P < 0.05$) DIAAS than roasted pork loin, which in turn had a greater ($P < 0.05$) DIAAS than raw pork loin. Results indicate that prepared pork loins can be considered excellent protein sources based on their DIAAS and these data make it possible to calculate DIAAS for meals containing commonly consumed pork loin products. Additionally, results of this research indicate that even for high-quality proteins, such as pork loin, correct preparation can improve DIAAS. Experiment 3 was conducted to determine DIAAS values for 10 different foods known to have different protein values: wheat bread, whey protein isolate, zein, sorghum flour, bovine collagen, black beans, pigeon peas, chick peas, roasted peanuts, and Kellogg's® All-Bran®. The second objective was to determine the variability among replications in the determination of the DIAAS values. Thirteen ileal-cannulated gilts were assigned to an incomplete 13 × 6 Latin square design with 13 diets and 6 periods. The 10 ingredients were used to formulate 10 different diets where each ingredient was the sole source of amino acids in the diet. Pigs on treatments 1 to 10 were fed the 10 diets containing the 10 food sources. Pigs on treatments 11, 12, and 13 were fed the whey protein isolate diet, the sorghum diet, and the pigeon pea diet, respectively. These extra replications enabled determination of intra-experiment variability. The SID for total amino acids was greater ($P < 0.05$) in toasted wheat bread and sorghum flour than in all other

proteins except for chickpeas. The SID for mean indispensable amino acids, mean dispensable amino acids and total amino acids was lower ($P < 0.05$) in All-Bran® than in all other proteins except roasted peanuts. The DIAAS was 0 for zein, bovine collagen, roasted peanuts, and All-Bran® for all reference ratios. Whey protein isolate had the greatest ($P < 0.05$) DIAAS for infants, followed by chickpeas, pigeon peas, sorghum flour, black beans, and toasted wheat bread, in descending order. Whey protein isolate had the greatest ($P < 0.05$) DIAAS for children (6 m to 3 y) followed by chick peas and pigeon peas. Black beans and sorghum flour had DIAAS values that were not different, but these values were greater ($P < 0.05$) than the DIAAS for toasted wheat bread. Whey protein isolate had the greatest ($P < 0.05$) DIAAS for older children (3 y and older), followed by chickpeas. Pigeon peas had a greater ($P < 0.05$) DIAAS than sorghum flour, which in turn had a greater ($P < 0.05$) DIAAS than black beans, and black beans had a greater ($P < 0.05$) DIAAS than toasted wheat bread. For DIAAS calculated for all 3 reference ratios, there were no differences between replications for whey protein isolate, sorghum, or pigeon peas. The DIAAS values determined in this experiment indicate that most legumes and cereal grain products tested in this experiment are not adequate as the sole sources of protein for humans. Results of this experiment demonstrate that the pig model is a consistent model for determination of amino acid digestibility and DIAAS determination even when disparate protein sources are used.

Key words: protein quality, DIAAS, PDCAAS, amino acids, digestibility, pigs

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CHAPTER 1: INTRODUCTION

Evaluation of protein as a nutrient began centuries ago (Munro, 1964). Nonetheless, characterization proteins in terms of their nutritive value and the optimization of their use in diets is still a highly debated topic (FAO, 1991). Proteins have been understood as compositions of individual amino acids for over a hundred years, and these individual amino acids have been recognized as distinct nutrients for nearly as long. In modern animal agriculture, most animals are fed on an amino acid basis (Fuller et al., 1989), with some system even accounting for the digestibility of individual amino acids (NRC, 2012). Despite this knowledge, in the field of human nutrition, most diets are still formulated based on protein.

Not all proteins are equal. Every protein has a specific composition of amino acids and not all amino acids in a food are bioavailable. As a result, formulation of diets on a protein basis is both imprecise and inaccurate. Accordingly, as understanding of protein in the diet has evolved, so too has its evaluation. A protein's value as a source of indispensable amino acids in the diet, its "protein quality", has become the primary focus (FAO 1991; 2013). The current gold standard for protein quality evaluation, protein digestibility corrected amino acid scoring (PDCAAS) has recently been challenged by a new system called digestible indispensable amino acid scoring (DIAAS; FAO; 2013).

There are several advantages to the DIAAS methodology over PDCAAS (many of which are discussed in later chapters), and as such the FAO has endorsed it as the successor to PDCAAS (FAO, 2013). However, substantial work is required before DIAAS can replace the PDCAAS, which has been the premier method for protein quality evaluation for over 25 years. As a result, there is a substantial database of PDCAAS values available. Additionally, years of use have proven that the PDCAAS system is both robust and effective. Therefore, the DIAAS

system must also undergo rigorous evaluation. Before DIAAS can be used in place of PDCAAS, a database of DIAAS values is needed so that DIAAS can be used for diet formulation. In addition, DIAAS determination needs to be proven consistent and repeatable.

Accurate evaluation of protein quality is of great value to nutritionists, but the true value lies in its use to address global malnutrition. It is estimated that as of 2016 there were 815 million people around the world suffering from malnutrition; this value is an increase from 2015 (FAO, 2017). Protein-energy malnutrition specifically is a significant concern. In developing countries, protein-energy malnutrition is estimated to account for over 56% of child deaths (Semba, 2016). In already marginalized populations, meeting amino acid requirements can be a matter of life or death. With this in mind, it is clear that improvements in protein quality evaluation is a matter of some urgency, and for these reasons, the DIAAS system needs to be adopted, implemented, and validated as soon as possible.

LITERATURE CITED

Munro, H. N. 1964. Historical introduction: the origin and growth of our present concepts of protein metabolism. In: H. N. Munro and J. B. Allison, ed., Mammalian protein metabolism volume 1. 1st ed. Academic Press, New York, New York. p. 1-29.

FAO. 1991. Protein quality evaluation. Report of a joint FAO/WHO expert consultation. FAO food and nutrition paper 51. Food and Agriculture Organization of the United Nations. Bethesda, 1991.

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. FAO food and nutrition paper 92. Food and Agriculture Organization of the United Nations. Rome, 2013.

FAO, IFAD, UNICEF, WFP, and WHO. 2017. The state of food security and nutrition in the world 2017. Building resilience for peace and food security. Food and Agriculture Organization of the United Nations. Rome, 2017.

Fuller, M., R. McWilliam, T. Wang, and L. Giles. 1989. The optimum dietary amino acid pattern for growing pigs: 2. Requirements for maintenance and for tissue protein accretion. Brit. J. Nutr., 62:255-267. doi:10.1079/BJN19890028

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC. doi:10.17226/13298

CHAPTER 2: EVALUATION OF THE NUTRITIONAL QUALITY OF PROTEINS: A REVIEW OF LITERATURE

INTRODUCTION

Protein was first described as a nebulous substance present in large concentration in animal tissues and in smaller concentrations in plant tissue in the mid-eighteenth century (Munro, 1985). However, it was not until 1772 that elemental N was isolated by Rutherford and 1816 that François Magendie demonstrated empirically the essentiality of N for life in dogs (Munro, 1964; 1985). Since their discovery, proteins have always been identified by their N content. Indeed, the term "protein" was coined by Dutch chemist Gerardus Mulder at the suggestion of Jac Berzelius in 1838 to represent the organic nitrogenous nuclei inherent to all nitrogenous compounds (Munro, 1985; Carpenter, 1994; Pencharz, 2012; Stipanuk, 2013). Despite Mulder's theories regarding the fundamental structure of all nitrogenous compounds being proven flawed, the term "protein" is still in use today (Munro, 1985), although it is used in a somewhat different sense.

A more contemporary definition for protein is as follows: "high molecular weight polyamides, consisting of one or more chains of amino acids, which fold into a form that gives…particular function" (Eastwood, 2003). Nearly 200 years after Mulder's original work, N remains central to the identity of protein. However, as research has progressed, the definition for protein has evolved to describe not only the structure of proteins, but also to include their inherent functionality, which is defined by both the amino acids composition of protein and the configuration (Eastwood, 2003; Pencharz, 2012). However, understanding the inherent nature of proteins to be dependent on intrinsic peptides and, therefore, amino acids was an advance that

took several decades. Although amino acids were known as hydrolytic components of protein as early as 1820 (Munro, 1964), the elucidation of a protein structure to consist of polypeptides that were in turn composed of amino acids was primarily the result of the separate, but related, work of 3 chemists near the turn of the 19th century: Emil Fischer, Albrecht Kossel, and Franz Hofmeister (Fruton, 1979). It was, however, Fischer who was the first to use the term "peptide" and "polypeptide", but it took several decades before the polypeptide hypothesis was fully accepted (Fruton, 1979).

Alongside advances in protein chemistry, and despite relative uncertainty regarding the ultimate chemical composition of proteins, research regarding the nutritional utilization of N continued. Nitrogen balance studies were conducted in dairy cattle as early as in 1839 by Jean-Baptiste Boussingault, and some of the first protein requirements were proposed by Lyon Playfair in 1865 (Munro, 1964; 1985). These requirements were based on Justus von Liebig's false assumption that protein was broken down to fuel work, and thus needed to be replenished (Munro, 1985). Despite his discredited assumptions regarding protein metabolism, Liebig was one of the first to acknowledge functional differences in the quality of proteins with his critique of gelatin in the early 1840's (Munro, 1964; 1985).

Gelatin continued to be used in protein research, and Kauffman conducted a N balance study on himself in 1905 and determined that he could maintain N balance if he consumed gelatin supplemented with Tyr, Cys, and Trp; this was, however, in direct contradiction to similar contemporary experiments conducted by Rona and Müller (Munro, 1964). Just one year later, however, Willcock and Hopkins were able to improve longevity and welfare of mice fed zein (a purified corn protein) with the supplementation of Trp, and in 1914, Osborne and Mendel determined that further addition of Lys to this mixture created a wholly adequate diet for mice

(Munro, 1964). It was this work that set the stage for a student of Mendel, William C. Rose, to begin to create diets in which protein could be replaced entirely by amino acids and to ultimately discover the final proteinogenic amino acid, Thr (McCoy et al., 1935; Munro, 1964). With the discovery of Thr, it became possible to formulate diets using only purified amino acids, and thus began an era of great progress in the understanding of individual amino acid requirements. The understanding of the relative value of a protein naturally resulted in the need for a more specific understanding of its component amino acids. In fact, just over a decade after the discovery of Thr, the first system for evaluation of proteins based on their amino acid composition was developed by Block and Mitchell (1946) who used a "chemical score" that was calculated using egg protein as the standard. The scoring of a protein and the use of egg as the standard highlighted the functional value of protein as a nutrient. Despite the existence of hundreds of amino acids in nature, only 20 amino acids can be coded for transcription by mRNA (Eastwood, 2003; Pencharz, 2012). This does not, however, imply that only 20 amino acids are found in body proteins, but simply that only these 20 amino acids can be transcribed; other required amino acids can be synthesized in-protein via post-translational modifications (Stipanuk, 2013). Because these 20 amino acids are the amino acids needed for the synthesis of all body prions, they are the only amino acids of major dietary concern in humans.

In a normal human diet, purified amino acids are rarely directly consumed. Typically, amino acids are provided in the diet as components of proteins. Whole proteins are subject to digestion whereupon they are hydrolyzed into smaller peptides, which may subsequently be further broken down into free amino acids that can enter the blood stream (Moughan and Stevens, 2013). The body is able to utilize these amino acids in various metabolic processes and, most notably, in protein synthesis.

Because amino acids are absorbed from the diet, as opposed to whole proteins, and because amino acids are the foundation of protein metabolism, the distinction between amino acids and protein is important. As the foundation of protein metabolism, i.e. synthesis and degradation, amino acids need to be treated as distinct nutrients, each with their individual requirement (FAO, 2013). Indeed, from a nutritional perspective the value of a protein in the diet is directly related to its amino acid composition (NRC, 2012). Consequently, there is no requirement for protein *per se* and protein intake may not always adequately reflect amino acid intake.

PROTEIN DEFICIENCY

Millions of children across the world depend on staple foods that are poor sources of indispensable amino acids (Semba, 2016a) and low serum concentrations of essential amino acids have been associated with stunting in children (Semba et al., 2016b). Protein-energy malnutrition has been calculated to account for approximately 56% of child deaths in developing countries (Pelletier et al., 1993; Semba, 2016a). However, protein-energy malnutrition is not limited to children in developing countries. Elderly individuals in developing countries are subject to high rates of protein malnutrition (Volkert and Sieber, 2011; Donini et al., 2013). Additionally, recent evidence indicates that the protein requirement for elderly individuals may currently be underestimated (Volkert and Sieber, 2011; Donini et al., 2013). It is for these reasons that the FAO sees this "as a matter of urgency". The potential complications from amino acid deficiency on health and welfare are serious, lasting, and can include death.

Protein deficiency is a worldwide phenomenon that has been researched extensively. However, malnutrition in the global sense is nearly always a multi-factorial issue. Therefore, isolating the precise numbers of individuals who suffer from protein malnutrition is complicated.

Despite progress in determining protein quality and malnutrition the incidence of malnutrition has been increasing (FAO, 2017). Though most of this increase is assumed to be caused by geopolitical conflicts, it is estimated that as of 2016 there were 815 million people around the world suffering from malnutrition (FAO, 2017).

In general, there are 3 main indicators of undernutrition: wasting (low weight for height), stunting (low height for age), and being underweight (low weight for age; Onís et al., 1993). These indicators are associated with protein-energy malnutrition (**PEM**) and more than one-third of the world's children suffer from PEM (Onís et al., 1993). Despite the lifelong requirement for amino acids in humans, protein deficiency is perhaps best characterized by its effects on infants and growing children due to the relatively higher amino acid requirements if expressed as concentration of diet. As a result, one of the earliest described and primary protein deficiency syndromes described throughout history is one that is nearly exclusive to children: "kwashiorkor" (Carpenter, 1994c).

Kwashiorkor was first official identified in Cicely Williams' publication in the Archives of Disease in Childhood (1933) after her first-hand experience with the disease in West Africa. The disease was limited to children of typically 1 to 2 y of age and presented itself with symptoms that included generalized edema, irritability, swollen abdomens, diarrhea, and flaky skin (Williams, 1933). Without treatment, the disorder was invariably fatal. Although Williams was correct in assuming the disease was related to the diet, it was not immediately clear at the time that kwashiorkor was caused by protein deficiency. In fact, the title of the article (A Nutritional Disease of Childhood Associated with a Maize Diet) clearly indicates her understanding of the nutritional etiology of the disease. However, when the evidence is viewed through modern eyes, the relationship becomes clearer. The name kwashiorkor was given to the

disease by the locals, which translates to “the deposed child” in English (Carpenter, 1994c). The name referred to the fact that most cases of kwashiorkor occurred in children once their mother had begun nursing another, younger child. Kwashiorkor progressed despite the fact that the children often began consuming solid foods, primarily grains and tubers like corn and cassava (Williams, 1933). These foods although high in energy, are low in amino acids. Combined with the lack of breast milk, children consuming these diets were severely lacking in indispensable amino acids.

Although identified nearly 100 years ago, kwashiorkor, or as it is now known, oedematous malnutrition, is still commonplace throughout the developing world (Manary et al., 2009). This is made all the more tragic in consideration of the fact that the cause of the disease is now well understood, highlighting the importance of using knowledge about protein quality in diet formulations.

HISTORICAL INTERPRETATIONS OF PROTEIN QUALITY

The concept of amino acids as individual nutrients was understood through the combined, but largely separate, work of Rose and Mitchell at the University of Illinois (Carpenter, 1994b). Most conventional feeding programs for pigs and poultry have been based on individual amino acids for over 40 years (Fuller et al., 1989), but this concept has not been utilized in the field of human nutrition. However, the importance of proteins in human nutrition has been recognized for at least 100 years and there have been a number of measures for protein quality used during the last century.

Protein Efficiency Ratio (PER)

One of the first methodologies for comparing proteins on a quantitative basis was developed approximately 100 years ago (Osborne et al., 1919; Carpenter, 1994d). Although, not

explicitly called the "Protein Efficiency Ratio" (**PER**) in their original publication, the basis for PER determination was established (Osborne et al., 1919). It was understood that growth was directly related to intake of protein, and it was accepted that by limiting the concentration of protein in food, growth was limited, which established the basis for evaluating proteins based on PER. By feeding rats a defined amount of a protein over a fixed amount of time, usually 4 weeks, and then evaluating weight gain relative to protein intake, the PER could be calculated (Friedman, 1996). For standardization purposes, adjusted PER could be determined if a control group of rats was fed a casein diet. A multiplier was used for both casein and the test protein to ensure that casein yielded a PER of 2.5, under the assumption that this adjustment would inhibit variation among rats from different colonies (Carpenter, 1994d). Though the official procedure for calculating PER required refinement over the following decades, particularly, by establishing a constant level of protein as approximately 10% of dry weight of the diet (Carpenter, 1994d; Gilani and Lee, 2003). However, PER was accepted as an official method for evaluating protein quality in the United States of America in 1965 (AOAC, 1965). As of 2018, PER is still the official methodology recommended for evaluation of protein quality by Health Canada (Health Canada, 1981).

The importance of the PER methodology to protein nutrition is difficult to overstate. It was a pioneering effort that enabled the evaluation of proteins on an objective, quantitative basis that strictly reflected the value of protein as a nutrient. Additionally, by using growth as a metric, calculation of PER offered insight into the value of proteins before the discovery of all indispensable amino acids and their specific requirements. However, because of the inability of PER to separate the protein requirement for growth and maintenance, PER falls short (Gilani and Lee, 2003). This is of concern because it prevents the proportionality of ratios from being

directly reflective of their comparative values (Gilani and Lee, 2003). The PER methodology also fails to account for the composition of the tissue in weight gained by the rat (Carpenter, 1994d). Additionally, although PER may be of great value to the formulation of murine diets, application of PER to human diets is more complex. Being based on the growth of the weanling rat, PER reflects a protein's ability to meet the requirements of the rat, not the human, and the differences in the requirements between rats and humans are not taken into account in the PER calculation.

In an attempt to account for the maintenance requirement, modifications to the PER protocol were made by creating the Net Protein Ratio (**NPR**). The NPR differs from PER in two primary ways. To make some estimation of the maintenance requirement, a control group of rats is fed a protein-free diet and the weight loss of these rats is then subtracted from the weight gain of rats on the protein treatment (Friedman, 1996; Gilani and Lee, 2003). Although this estimation is advantageous, it makes the assumption that the protein required to prevent weight loss is equivalent to the protein required for maintenance (Gilani and Lee, 2003). A second change that NPR methodology adds is that the experimental period is reduced from 4 weeks to 2 weeks (Gilani and Lee, 2003). In a further attempt to address shortcomings, Relative Net Protein Ratios (**RNPR**) were developed. To calculate RNPR, the NPR of a protein was divided by the NPR of a reference protein and expressed as a proportion of 100 (Gilani and Lee, 2003). This adaptation allowed for values to have increased precision compared with PER values and created values that were proportional to each other (Gilani and Lee, 2003).

Biological Value of Protein (BV) and Net Protein Utilization (NPU)

Rather than the use of growth as a metric for protein quality, other systems used N balance. The concept of BV was first explored in humans by Karl Thomas who described it as

the amount of N that was retained, i.e. not excreted in urine, from the consumption of a food protein assuming the diet met energy requirements (Carpenter, 1994d). However, the technique was more rigorously explored in rats by H. H. Mitchell at the University of Illinois who also fed N-free diets to enable determination of endogenous losses (Carpenter, 1994d). Using these values, Mitchell calculated BV using the following equation:

$$BV\ (\%) = [\{[Ni - (Nf + Nu)] - [Ni_f - (Nf_f + Nu_f)]\} / [Ni - (Nf - Nf_f)]] \times 100,$$

where BV is the biological value of the protein in the diet (%), Ni is the intake of N on the proteinaceous diet, and Nf is the fecal output of N on the proteinaceous diet, Nu is the urine output of N on the proteinaceous diet, Ni_f is the intake of N on the N-free diet, and Nf_f is the fecal output of N on the N-free diet, and Nu_f is the urine output of N on the N-free diet (Carpenter, 1994d; Friedman, 1996).

In a further attempt to modify BV to reflect not only absorption of N, but to also reflect N utilization, NPU was proposed, which is calculated as follows:

$$NPU\ (\%) = \{[Ni - (Nf - Nf_f)] / Ni\} \times BV$$

(Carpenter, 1994d; Friedman, 1996). In this way, N efficiency is not calculated based on digested N. Evaluating digestibility is an important consideration for the determination of a protein's quality, and in many ways digestibility can be used as an approximation for bioavailability (Stein et al., 2007a; 2007b).

INTAKE AND DIGESTIBILITY

Digestibility of Protein

Intake is only one component of nutrition. Bioavailability of nutrients is technically quite difficult to measure (Stein et al., 2007a). Therefore, an estimation for bioavailability is required. One such approximation for bioavailability that is nearly universally used in animal nutrition is

digestibility (Stein et al., 2007a; 2007b). Digestibility describes the difference between intake and output of a certain nutrient (Stein et al., 2007a; 2007b). In effect, the disappearance of a certain nutrient in the gastro-intestinal tract is assumed to reflect the absorption of that nutrient (Nyachoti and Stein, 2005; Stein et al., 2007a; 2007b).

Total Tract Digestibility

There are several ways to calculate digestibility of a nutrient. The simplest measure for digestibility is total tract digestibility. Total tract digestibility is by definition the fecal excretion of a nutrient subtracted from the intake of the nutrient. The difference divided by intake multiplied by 100 results in a digestibility value that is commonly reported as a percentage (Stein et al., 2007a). However, one of the problems with this approach is that there is no consideration for endogenous secretions of protein into the gastrointestinal tract. These secretions can originate from desquamated cells, mucins, enzymes, and bile acids (Nyachoti et al., 1997; Moughan, 2003). Any of these secretions into the gut results in decreased digestibility values. In animal nutrition, total tract digestibility that is calculated without consideration for endogenous secretions is called apparent total tract digestibility (**ATTD**; NRC, 2012). If the quantity of endogenous secretions can be determined, it can be incorporated into the calculation for digestibility, counted against the output, and then give a more accurate representation of the actual digestibility of the nutrient. This digestibility is called the standardized total tract digestibility (**STTD**; NRC, 2012).

Protein Digestibility Corrected Amino Acid Scoring (PDCAAS)

A meeting held in November of 1980 by the Codex Committee on Vegetable Proteins (**CCVP**) first began the official discussion regarding the inadequacy of PER to reflect protein quality and in their second meeting two years later, RNPR was considered as a potential

alternative (FAO, 1991). However, at their 3rd and 4th sessions, in 1984 and 1987, respectively, the CCVP suggested amino acid scores as an improved method, but acknowledged that both *in vitro* methods for determining protein quality and amino acid concentration determination methodology were lacking (FAO, 1991). At the CCVP's 5th session in 1989, the amino acid requirement data published for 2 to 5 y olds by a joint WHO/FAO/UNU venture were endorsed (WHO, 1985). The CCVP also recommended that a system utilizing amino acid scores corrected by amino acid requirements be created, however, in recognition of any such system's potentially far-reaching implications, they recommended a joint FAO/WHO Expert Consultation be held (FAO, 1991; 2013). Subsequently, in December of 1989, an FAO/WHO Expert Consultation reviewing protein quality evaluation was held (FAO, 1991; 2013). The primary outcome of this meeting was the development of a new system for evaluation of protein quality: PDCAAS.

The PDCAAS system utilized an *in vivo* rat assay to evaluate protein quality (FAO, 1991). First, an amino acid score is calculated for the test protein based on the lowest amino acid ratio calculated using the amino acid requirements established by the FAO/WHO/UNU (WHO, 1985; FAO, 1991). Ratios were determined by dividing the concentration in mg of each indispensable amino acid in 1 g of the test protein by the concentration of that same amino acid in 1 g of the reference pattern (WHO, 1985; FAO, 1991). Rats would be fed diets with a test protein formulated to 10% CP or a low-protein/protein-free diet and fecal N would be measured from both groups (FAO, 1991). Fecal N from rats fed the low or no protein diets was assumed to represent "metabolic nitrogen" or the basal endogenous N secretions (FAO, 1991). Using these values combined with intake, true digestibility of N could be calculated as follows:

$$TD\ (\%) = [Ni - (Nf - Nf_{f)} / Ni] \times 100,$$

where TD is the true digestibility of N, Ni is the intake of N, Nf is fecal N of rats fed proteinaceous diets, and Nf_f is the fecal N of rats fed the low or no protein diets (FAO, 1991). By then multiplying the lowest amino acid ratio (amino acid score) by the true digestibility of N and multiplying that by 100, PDCAAS is determined.

In many ways, the PDCAAS system was an improvement over the conventional methods for protein quality evaluation. It utilizes an economical, easily reproducible, *in vivo* assay that emphasized the importance of each indispensable amino acid as a distinct nutrient (FAO, 1991). Additionally, its amino acid focus enabled the possibility for the evaluation of diet mixtures and complementary ingredients (FAO, 1991; 2013). For these reasons, PDCAAS was the gold standard for protein quality evaluation for more than 20 years. However, although the measurement of fecal N digestibility can be made with relative ease, there are certain complications that arise from the use of fecal N digestibility as an indicator for amino acid digestibility.

Ileal Digestibility

The total tract digestibility of a nutrient can, technically, be measured in humans. In fact, the digestibility of protein in humans is often described using a measure of total tract digestibility. However, because proteins are composed of amino acids, total tract digestibility can be an oversimplification. In pigs, humans, and all other animals, amino acid absorption takes place entirely in the small intestine (Moughan et al., 2003). However, fecal content contains proteins that are synthesized by microbes in the hindgut and other non-dietary proteins. As a consequence, calculation of the ATTD or STTD of proteins will not result in an accurate estimate of protein digestion. However, if protein and amino acids that are leaving the small intestine are quantified and subtracted from the intake, the influence of the hindgut microbes is

avoided. The correlation between deposited protein and ileal digestible amino acids is greater than the correlation with fecal digestible protein and amino acids (Just et al., 1985; Leibholz, 1985) Therefore, to more accurately determine bioavailability of protein/amino acids, measurements need to be taken before the hindgut, that is, at the terminal ileum.

Eliminating the effect of hindgut fermentation of N on digestibility can and has been addressed in several ways in different species. In poultry, cecectomization is performed to minimize the effect of hindgut fermentation on protein metabolism and excreta output is presumed to represent digestibility of protein (Parsons et al., 1985). In humans, although fecal sampling is common, ileostomates have been used for collection of ileal effluent samples that may better reflect amino acid digestibility (Rowan et al., 1994; Darragh and Hodgkinson, 2000; Gaudichon et al., 2002). In limited cases, naso-ileal tubing has also been used to sample terminal ileal contents (Gaudichon et al., 2002; DeGlaire et al., 2009; Miner-Williams et al., 2014). However, this technique has several drawbacks in that it is relatively invasive, technically challenging, requires healthy volunteers, and is extremely costly (Gaudichon et al., 2002; DeGlaire et al., 2009; Miner-Williams et al., 2014). Additionally, studies using naso-ileal tubing are often acute-feeding studies and, therefore, do not account for adaptation to the diet (Miner-Williams et al., 2014). Ileostomy procedures have, in theory, been conducted in pigs (Laplace et al., 1994), and several studies have been conducted using pigs that have undergone ileo-rectal anastomoses to determine disappearance of amino acids at the end of the ileum (Yin et al., 1993; Laplace et al., 1994; Mariscal-Landín et al., 1995). All of these techniques can mitigate or eliminate effects of hindgut metabolism on the digestibility of amino acids and protein, but there are several drawbacks to using these approaches.

Resection of the gastrointestinal tract, in any form, alters the natural physiology of any animal and introduces sources of potential variation from the metabolism of a typical subject. In addition, complete removal of the hindgut can alter the transit rate and time of digesta, and therefore, may change digestibility of ingredients and overall metabolism (Laplace, 1981; Laplace et al., 1994; Miner-Williams et al., 2014). In ileostomates, the removal of the colon can influence microbial infiltration of the ileum, with ileal effluent from ileostomates having counts of microbial organisms that are 80-fold greater than that of standard ileal samples (Rowan et al., 1994). Alterations in the microbiome may have significant effects on metabolism (Miner-Williams et al., 2014). In the pig, it has been estimated that up to 16% of the gross energy of the diet can be recovered in the large intestine (Shi and Noblet, 1993). Removal of the large intestine, therefore, alters energy balance of the animal and may introduce protein-energy interactions (Laplace et al, 1994). Removal of the large intestine involves removal of a substantial mass of metabolically active tissue, and therefore, subjects of these procedures may have different maintenance requirements compared with intact animals. The various anastomotic techniques in animal models were developed as alternatives to some of the initial cannulation techniques (Yin et al., 1993). The re-entrant cannulation technique, although effective, was technically challenging to implement and difficult to use in long-term studies in pigs (Yin et al., 1993). However, improvements in the cannulation technique, in particular the use of "T-cannulas", made surgeries easier to perform, minimized invasiveness, and were effective long-term implants (Furuya et al., 1974; Stein et al., 1998; 2007b).

The precise surgical techniques are explained in detail by Furuya et al. (1974) and have been modernized by others (Decuypere et al., 1977; Gargallo and Zimmerman, 1980; Stein et al., 1998). In brief, an incision is made in the terminal ileum of the pig and the T-cannula is then

inserted and sutured into place. The cannula is externalized via a separate, more dorsal incision and capped to prevent leakage. After a brief period of recovery, generally 7 to 10 days, the incisions are healed, the cannulas are secure, and ileal effluent samples can be collected at will (Stein et al., 1998).

Time has proven that T-cannulation is effective, robust, and well accepted. Using this technique, hundreds of experiments have been conducted across the globe to determine ileal digestibility of amino acids in many feed ingredients. Indeed, T-cannulation derived ileal digestibility values have been the gold standard for formulation of swine diets for decades (NRC, 1998; 2012). However, much like other measures of digestibility, there have been improvements within the ileal standard, as well.

Apparent Ileal Digestibility (AID)

Similar to the equations by which ATTD is determined AID can be determined:

$$\text{AID (\%)} = [AA_i - AA_o / AA_i] \times 100,$$

where AID is the apparent ileal digestibility, AA_i is the amino acid intake from the diet, and AA_o is the ileal ouput of amino acids (Stein et al., 2007b). However, unlike ileo-rectal anastomotic procedures, only a portion of ileal digesta are collected when using the cannulation technique and, therefore, indigestible markers need to be included in the diet to calculate digestibility:

$$\text{AID (\%)} = [1 - (AA_{digesta} / AA_{diet}) \times (M_{diet} / M_{digesta})] \times 100,$$

where $AA_{digesta}$ is the amino acid concentration in the ileal digesta, AA_{diet} is the amino acid concentration from the diet, M_{diet} is the concentration of the indigestible marker in the diet, and $M_{digesta}$ is the concentration of the indigestible marker in the ileal digesta (Stein et al., 2007b). Values for AID are arguably more relevant to protein deposition of the animal, however, despite this there are disadvantages with any of the apparent measure of digestibility.

One of the primary concerns with AID values are their lack of additivity when using mixtures of ingredients (Stein et al., 2005). The reason for the lack of additivity is that diet amino acid level will affect the ileal outflow of amino acids (Fan et al., 1994) and as the level of amino acids in the diet increases, the relative contribution of endogenous losses to the total amino acid outflow is reduced. To overcome these obstacles, efforts were made to quantify endogenous amino acid losses, which consist of proteins synthesized by the animal, secreted into the small intestine and are not reabsorbed before reaching the large intestine (Hodgkinson and Moughan, 2000). These endogenous amino acid losses can be further divided into two categories: basal endogenous losses or specific endogenous losses (Nyachoti et al., 1997; Jansman et al., 2002; Stein et al., 2007b). Basal endogenous losses are defined as the minimum quantities of amino acids that are lost by the animal and these losses are inevitable and completely independent from specific diet effects (Nyachoti et al., 1997; Jansman et al., 2002; Stein et al., 2007b). Specific losses, on the other hand, are those that are directly related to the diet and its composition (Stein et al., 2007b). These losses can vary among food and feed ingredients and are related to specific characteristics of the diet such as fiber-content and the presence of anti-nutritional factors (Stein et al., 2007b).

True Ileal Digestibility (TID)

In an effort to account for all endogenous losses, calculation of TID was suggested (Schumann et al., 1986). In this system, the total endogenous losses of amino acids are subtracted from the ileal outflow in an attempt to more accurately represent what is sometimes referred to in the literature as “real” digestibility. The equation is a modification of that for AID:

$$\text{TID (\%)} = \text{AID} + [(\text{total } AA_{end} / AA_{diet)} \times 100],$$

where total AA_{end} represents the sum of basal and specific endogenous losses. This calculation effectively incorporates the metabolic cost of consuming a certain feed ingredient into the equation, however, specific endogenous losses are variable based upon ingredient composition of the diet. In practice, specific endogenous losses can be challenging to determine empirically. However, estimating basal endogenous losses enjoys a well-established protocol involving the use of protein-free or low-protein diets (Stein et al, 2007b). There are minimal differences in the basal endogenous losses when using either low-protein or protein-free diets (Jansman et al., 2002), and formulation of protein-free diets has become formalized (Stein et al., 2007).

Standardized Ileal Digestibility (SID)

Utilizing the ability to determine the basal endogenous losses with relative certainty, one can calculate SID as follows:

$$SID\ (\%) = AID + [(\text{basal } AA_{end} / AA_{diet)} \times 100],$$

where basal AA_{end} represents the basal endogenous losses determined via the feed of a low or no-protein diet (Stein et al, 2007b). The value of the SID system is that it removes the variation that occurs in AID values when varying protein levels of an ingredient are used (Stein et al, 2007b), and it enables additivity of values from individual ingredients when they are included in a mixed diet which is of practical importance to diet formulation (Stein et al., 2005). Because only the basal endogenous losses are subtracted from the ileal output when SID values are calculated, the specific endogenous losses are counted against each ingredient individually, which results in ingredients causing high specific endogenous losses of amino acids to be penalized in diet formulation. As a result, values for SID of amino acids have become available for nearly all feed ingredients fed to swine and SID digestibility is the gold standard for the formulation of swine diets throughout the world (NRC, 2012). The robustness of the SID system

resulted in this system not only being used to evaluate the availability of amino acids in an ingredient, but also to describe the requirements for amino acids in pigs (NRC, 1998; 2012). Decades of titration studies to determine amino acid requirements have enabled requirements for amino acids by pigs to be described on an SID basis, divided by both age and gender (NRC, 2012).

DIGESTIBLE INDISPENSABLE AMINO ACID SCORING (DIAAS)

In contrast to the accuracy, precision, and depth with which ileal digestible amino acid requirements in pigs have been determined, there is a dearth of these values for humans, despite the understanding that requirements based on ileal digestible amino acid values are ideal. However, there are various reasons for this difference; most importantly are ethical concerns and expenses of conducting research using human subjects versus animal subjects. To overcome some of these challenges modeling the human has proven a viable alternative (FAO, 2013).

The pig has been explored as a model for human protein nutrition on several occasions (Rowan et al., 1994; Moughan, 2003; Deglaire et al., 2009; FAO, 2013), and is generally considered the best available model (Rowan et al., 1994; Moughan, 2003; Deglaire et al., 2009; FAO, 2013). For these reasons, an ileal digestibility model using pigs was suggested as the successor for the PDCAAS system by an FAO Expert Consultation in 2011 (FAO, 2013). The DIAAS systems involves the following calculation:

DIAAS (%) = [(mg of digestible dietary indispensable amino acid in 1g of test protein) / (mg of the same amino acid in 1 g of reference protein) × 100].

DIAAS versus PDCAAS

The calculation to determine DIAAS values is similar to the calculation used to calculate PDCAAS with the exception that ileal digestibility values for each indispensable amino acid are

used. This change is arguably the most significant change from PDCAAS. Unlike PDCAAS, each of the indispensable amino acid in the reference protein are treated as an individual nutrient and are, in turn, each afforded an empirically determined digestibility value (FAO, 2013). Through this change, the dubious assumption that fecal N digestibility values can represent that of all amino acids is completely avoided. Additionally, the change from fecal to ileal digestibility more accurately represents the biological reality of amino acid absorption because amino acid absorption is completed before the end of the ileum (Rowan et al, 1994; Gaudichon et al., 2002; Deglaire et al., 2009; FAO, 2013).

However, other DIAAS specific changes include a revised amino acid requirement pattern/reference protein (FAO, 2013). Not only were the requirements refined, but also expanded into 3 categories: birth to 6 m, 6 m to 3 y, and 3 y and older (FAO, 2013). Using these new reference patterns, 3 DIAAS values can be generated for a single protein, each reflecting its value as an amino acid source for an individual in each of these 3 age brackets. In addition, unlike PDCAAS, DIAAS values are not truncated at 1.00 or 100% (FAO, 2013). This change allows combined DIAAS values to be used to evaluate diets with more than one protein source (FAO, 2013). In consideration of the aforementioned points, the FAO made several decisions at the 2011 Expert Consultation. The first was that DIAAS was the recommended methods for dietary protein assessment for regulatory purposes, but until a substantial database with SID values for proteins commonly consumed by humans was established, fecal N digestibility values should be used (FAO, 2013). Secondly, it is recommended that ileal digestibility data for human foods should be preferentially determined using the pig model (FAO, 2013).

More recently, it was also concluded that PDCAAS has a tendency to underestimate the quality of high-quality proteins and overestimate the quality of low-quality proteins (Rutherfurd

et al., 2015). Although perhaps seemingly an innocuous flaw, this potential error may have implications for individuals consuming primarily dietary sources of protein of lower quality. The advantage of using the DIAAS system, therefore, is greatest in assessing the adequacy of protein intake in populations typically consuming proteins of low quality and in low quantities.

This most recent critique of the PDCAAS technique highlights the implications of a better understanding of protein quality. Accurate estimation of protein quality will enable an accurate formulation of diets that will meet the requirements of individuals. Although, to much of the developed world, protein quality may not be a critical nutritional issue, to large segments of the global population meeting daily amino acid requirements is not only difficult, it is in many cases impossible.

DIAAS OF PROTEINS

In recent years, DIAAS values have been determined in several proteins using rats or pigs. Based on the recommendations of the FAO, the pig is the preferred model, whenever it is possible to use pigs. Nonetheless, several published values for DIAAS of various proteins determined using rats have been published (Rutherfurd et al., 2014; Nosworthy et al., 2017a; 2017b; 2018). However, methodologies within these experiments differed. The 2014 study by Rutherfurd et al. euthanized the rats and collected ileal digesta samples to determine the digestibility of individual amino acids for calculation of DIAAS values. However, all studies conducted by Nosworthy et al., used fecal digestibility of N to determine DIAAS (Nosworthy et al., 2017a; 2017b; 2018). Although the FAO recommendations do allow for the use of fecal crude protein digestibility when amino acid digestibility data is not available, it also specifies that *in* vivo assays to determine true ileal amino acid digestibility should be conducted when new foods are being assayed (FAO, 2013). All studies conducted by Nosworthy et al. (2017a; 2017b;

2018) were conducted using previously unstudied legume products. Accordingly, the DIAAS values presented by their data should have been determined using ileal digestibility values for amino acids. At least one study determined DIAAS via an *in vitro* gastrointestinal model, however, as this methodology has yet to be validated by comparisons with *in-vivo* data, results should be interpreted cautiously (Havenaar et al., 2016).

Values for DIAAS are available for several cereal grains including corn, barley, oats, oat protein concentrate, rice, rye, sorghum, and wheat (Cervantes-Pahm et al., 2014; Rutherfurd et al., 2015; Mathai et al., 2017; Abelilla et al., 2018). Although the DIAAS values of these proteins are varied, none but rolled oats scored greater than the minimum recommended score of 75 for a "good" source of protein made by the FAO (FAO, 2013; Cervantes-Pahm et al., 2014; Rutherfurd et al., 2015; Abelilla et al., 2018). Cooked rolled oats, however, received a lower DIAAS (Rutherfurd et al., 2015). Additionally, legume-based ingredients such as soy flour, soy protein isolates, and roasted peanuts have been evaluated for their DIAAS values (Rutherfurd et al., 2015). In general, the soy products have met the FAO standard for "good" proteins (Rutherfurd et al., 2015). Several animal proteins, both dairy and meat-based, have also had their DIAAS evaluated (Rutherfurd et al., 2015; Bindari et al., 2018). Dairy proteins have generally scored highly Rutherfurd et al., 2015), however, the porcine and bovine tissue hydrolysates tested by Bindari et al. (2018) were of varying protein quality. In the study conducted by Rutherfurd et al., DIAAS values for animal proteins were higher than those for plant proteins due to higher amino acid digestibility values and the amino acid compositions of the animal proteins (Rutherfurd et al., 2015). The DIAAS system is better suited for evaluation of mixtures of ingredients than alternative methods, yet to date, no studies have been published that evaluate diets or ingredient mixtures for their DIAAS values. However, Rutherfurd et al. (2015)

calculated values for a combination of a corn-based breakfast cereal and milk protein concentrate that did have determined DIAAS values to emulate a common breakfast meal in the developed world. Results indicated that the two proteins complemented each other well.

CONCLUSION

Though the beginnings of protein quality evaluation began over a century ago, significant and meaningful progress has been steadily made. In continued efforts to create robust, reliable, and relevant methodologies, several iterations have been proposed. The DIAAS system represents the most recent of such iterations and signifies an effort to both modernize and refocus discussions of protein quality on amino acids and their physiological relevance. Unlike its predecessors, DIAAS represents several paradigm shifts in that it changes the primary animal model used for protein quality determination, it treats each indispensable amino acid as a distinct nutrient, and that it accounts for each amino acid's individual absorption.

However, before DIAAS can be fully accepted as an official methodology for determination of protein quality, DIAAS itself must undergo rigorous evaluation. For DIAAS to replace the current methodologies, DIAAS must first prove its consistency There remains a need for the generation of a database of DIAAS values for various proteins in order to facilitate the adoption of DIAAS values for diet formulation. Furthermore, the repeatability, precision, and accuracy of DIAAS values has yet to be fully determined. Nonetheless, after adequate validation, DIAAS values will represent the forefront methodology for evaluation of protein quality in foods.

LITERATURE CITED

Abelilla, J. A., Y. Liu, and H. H. Stein. 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

AOAC. 1965. Official Methods of Analysis. 10th ed. Association of Official Analytical Chemists, Washington, DC.

Bindari, Y. R., H. N. Lærke, and J. V. Nørgaard. 2018. Standardized ileal digestibility and digestible indispensable amino acid score of porcine and bovine hydrolyzates in pigs. J. Sci. Food Agric. 98:2131-2137. doi:10.1002/jsfa.8697

Block, R. J., and H. H. Mitchell. 1940. The correlation of the amino acid composition of proteins with their nutritive value. Nutr. Abstr. Rev. 16:249-278. doi:10.1016/0009-8981(60)90081-4

Carpenter, K. J. 1994a. Protein discovered and enthroned, 1838-1845. In: K. J. Carpenter, editor, Protein and Energy: A Study of Changing Ideas in Nutrition. 1st ed. Cambridge University Press, Cambridge, UK. p. 40-54.

Carpenter, K. J. 1994b. Vitamins and Amino acids, 1910-1950. In: K. J. Carpenter, editor, Protein and Energy: A Study of Changing Ideas in Nutrition. 1st ed. Cambridge University Press, Cambridge, UK. p. 119-141.

Carpenter, K. J. 1994c. Protein Deficiency as a Third World Problem. In: K. J. Carpenter, editor, Protein and Energy: A Study of Changing Ideas in Nutrition. 1st ed. Cambridge University Press, Cambridge, UK. p. 142-160.

Carpenter, K. J. 1994d. Appendix B: The Measurement of Protein Quality. In: K. J. Carpenter, editor, Protein and Energy: A Study of Changing Ideas in Nutrition. 1st ed. Cambridge University Press, Cambridge, UK. p. 238-244.

Cervantes-Pahm, S. K., Y. Liu, and H. H. Stein. 2014. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br. J. Nutr. 111:1663-1672. doi: 10.1017/s0007114513004273.

Darragh, A. J., and S. M. Hodgkinson. 2000. Quantifying the digestibility of dietary protein. J. Nutr. 130:1850S-1856S. doi:10.1093/jn/130.7.1850S

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

Donini, L. M., E. Poggiogalle, M. Piredda, A. Pinto, M. Barbagallo, D. Cucinotta, and G. Sergi. 2013. Anorexia and eating patterns in the elderly. PLoS One. 8:e63539. doi:10.1371/journal.pone.0063539

Eastwood, M. 2003. Principles, amino acid, and proteins. In: M. Eastwood, editor, Principles of human nutrition. 2nd ed. Blackwell Publishing Company, Malden, MA. p. 151-178.

Fan M. Z., W. C. Sauer, R. T. Hardin, and K. A. Lien. 1994. Determination of apparent ileal amino acid digestibility in pigs: Effect of dietary amino acid level. J. Anim. Sci. 72:2851-2859. doi:10.2527/1994.72112851x

FAO. 1991. Protein quality evaluation. Report of a joint FAO/WHO expert consultation. FAO food and nutrition paper 51. Food and Agriculture Organization of the United Nations. Bethesda, 1991.

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. FAO food and nutrition paper 92. Food and Agriculture Organization of the United Nations. Rome, 2013.

FAO, IFAD, UNICEF, WFP, and WHO. 2017. The state of food security and nutrition in the world 2017. Building resilience for peace and food security. Food and Agriculture Organization of the United Nations. Rome, 2017.

Friedman, M. 1996. Nutritional value of proteins from different food sources. A review. J. Agric. Food Chem. 44:6-29. doi:10.1021/jf9400167

Fruton, J. S. 1979. Early theories of protein structure. Ann. N. Y. Acad. Sci. 325:1-20. doi:10.1111/j.1749-6632.1979.tb14125.x

Fuller, M., R. McWilliam, T. Wang, and L. Giles. 1989. The optimum dietary amino acid pattern for growing pigs: 2. Requirements for maintenance and for tissue protein accretion. Brit. J. Nutr., 62:255-267. doi:10.1079/BJN19890028

Furuya, S., S. Takahashi, and S. Omori. 1974. The establishment of T-piece cannula fistulas into the small intestine of the pig. Jpn. J. Zootech. Sci. 45:42-44. doi:10.2508/chikusan.45.42.

Gargallo, J. and D. R. Zimmerman. 1980. A simple intestinal cannula for swine. Am. J. Vet. Res. 41:618-619.

Gaudichon C., C. Bos, C. Morens, K. J. Petzke, F. Mariotti, J. Everwand, R. Benamouzig, S. Daré, D. Tomé, C. C. Metges. 2002. Ileal losses of nitrogen and amino acids in humans and their importance to the assessment of amino acid requirements. Gastroenterology. 123:50-59. doi:10.1053/gast.2002.34233

Gilani, G. S., and N. Lee. 2003. Protein | Quality. In: B. Caballero, ed. Encyclopedia of Food Sciences and Nutrition. 2nd ed. Academic Press, Cambridge, MA. p. 4847-4854.

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

Health Canada. 1981. Determination of protein rating. Health Canada's Health Protection Branch Method FO-1.

Hodgkinson, S. M., and P. J. Moughan. 2000. Amino acids - the collection of ileal digesta and characterization of the endogenous component. In: P. J. Moughan, M. W. A. Verstegen, and M. I. Visser-Reyneveld, ed. Feed Evaluation. Principles and Practice. Wageningen Academic publishers, Wageningen, the Netherlands. P. 57-76.

Jansman, A. J. M., W. Smink, P. van Leeuwen, and M. Rademacher. 2002. Evaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. Anim. Feed Sci. Technol. 98:49-60. doi:10.1016/S0377-8401(02)00015-9

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

Laplace, J. P. 1981. The transit of digesta in the different parts of the digestive tract of the pigs. In: A. E. Harper and G. K. Davis, ed. Nutrition in health and disease and international development. Progress in clinical and biological research. Alan Liss, New York. Vol. 77. p. 847-872.

Laplace, J. P., W. B. Souffrant, U. Hennig, E. Chabeauti, and C. Fevrier. 1994. Measurement of precaecal dietary protein and plant cell wall digestion in pigs; comparison of four surgical

procedures for ileo-rectal anastomosis. Livest. Prod. Sci. 40:313-328. doi:10.1016/0301-6226(94)90098-1.

Leibholz, J. 1985. An evaluation of total and digestible lysine as a predictor of lysine bioavailability in protein concentrates for young pigs. Br. J. Nutr. 53:615-624. doi:10.1079/BJN19780091

Manary, M. J., G. T. Heikens, and M. Golden. 2009. Kwashiorkor: more hypothesis testing is needed to understand the aetiology of oedema. Malawi Med. J. 21:106-107.

Mariscal-Landín, G., B. Sève, Y. Colléaux, and Y. Lebreton. 1995. Endogenous amino nitrogen collected from pigs with end-to-end ileorectal anastomosis is affected by the method of estimation and altered by dietary fiber. J. Nutr. 125:136-146. doi:10.1093/jn/125.1.136

McCoy, R. H., C. E. Meyer, and W. C. Rose. 1935. Feeding experiments with mixtures of highly purified amino acids. viii. isolation and identification of a new essential amino acid*. J. Biol. Chem. 112:283-302.

Moughan, P. J. 2003. Amino acid availabaility: aspects of chemical analysis and bioassay methodology. Nutr. Res. Rev. 16:127-141. doi:10.1079/NRR200365

Moughan, P. J. and B. R. Stevens. 2013. Digestion and absorption of protein. In: M. H. Stipanuk and M. A. Caudill, ed. Biochemical, Physiological, and Molecular Aspects of Human Nutrition. 3rd ed. Elsevier Saunders, St. Louis, MO. p. 163-178.

Munro, H. N. 1964. Historical introduction: the origin and growth of our present concepts of protein metabolism. In: H. N. Munro and J. B. Allison, ed., Mammalian protein metabolism volume 1. 1st ed. Academic Press, New York, New York. p. 1-29.

Munro, H. N. 1985. Historical perspective on protein requirements: objectives for the future. In: K. Baxter and J. C. Waterlow, ed., Nutritional adaptation in man. 1st ed. John Libbey & Company Limited, London, England. p. 155-168.

Nosworthy, M. G., A. J. Franczyk, G. Medine, J. Neufeld, P. Appah, A. Utioh, P. Prohlich, and J. D. House. 2017a. Effect of processing on the *in vitro* and *in vivo* protein quality of yellow and green split peas (Pisum sativum). J. Agric. Food Chem. 65:7790-7796. doi:10.1021/acs.jafc.7b03597

Nosworthy M.G., J. Neufeld, P. Frohlich, G. Young, L. Malcolmson, J. D. House. 2017b. Determination of the protein quality of cooked Canadian pulses. Food Sci. Nutr. 5:896-903. doi:10.1002/fsn3.473

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

Nyachoti C. M., C. F. M. de Lange, and H. Schulze. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: a review. Can. J. Anim. Sci. 77:149-163. doi:10.4141/A96-044

Nyachoti, C. M. and H. H. Stein. 2005. Bioavailability: amino acids. Encyclopedia of Animal Science. Marcel Dekker Inc.

NRC. 1998. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington DC. doi:10.17226/13298

Onís, M. de, C. Monteiro, J. Akré, and G. Clugston. 1993. The worldwide magnitude of protein-energy malnutrition: an overview from the WHO Global Database on Child Growth. Bull. World Health Organ. 71:703-712.

Parsons, C. M. 1985. Influence of caecectomy on digestibility of amino acids by roosters fed distillers' dried grains with solubles. J. Agric. Sci. 104:469-472. doi:10.1017/S0021859600044178

Pelletier, D. L., E. A. Frongillo Jr., and J.-P. Habicht. Epidemiologic evidence for a potentiating effect of malnutrition on child mortality. Am. J. Public Health. 83:1130-1133. doi:10.2105/AJPH.83.8.1130

Pencharz, P. B. 2012. Protein and amino acids. In: J. W. Erdman, Jr., I. A. Macdonald, S. H. Zeisel, ed. Present knowledge in nutrition. 10th ed. John Wiley & Sons, Inc, Ames, IA. p. 69-82.

Rowan, A. M., P. J. Moughan, M. N. Wilson, K. Maher, and C. Tasman-Jones. 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. 71:29-42. doi:10.1079/BJN19940108

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

Schumann, B., W. B. Souffrant, and G. Gebhardt. 1986. Nitrogen and amino acid absorption in the small intestine of growing pigs. 2. Apparent and true crude protein digestibility and amino acid absorption to the end of the small intestine. Arch. Tierernahr. 36:491-498.

Semba, R. D. 2016. The rise and fall of protein malnutrition in global health. Ann. Nutr. Metab. 69:79-88. doi:10.1159/000449175

Semba R.D., M. Shardell, F. A. Sakr Ashour, R. Moaddel, I. Trehan, K. M. Maleta, M. I. Ordiz, K. Kraemer, M. A. Khadeer, L. Ferrucci, and M. J. Manary. 2016. Child stunting is associated with low circulating essential amino acids. EBioMedicine. 6:246–252. doi:10.1016/j.ebiom.2016.02.030.

Shi, X. S., and J. Noblet. 1993. Contribution of the hindgut to digestion of diets in growing pigs and adult sows: effect of diet composition. Livest. Prod. Sci. 34:237-252. doi:10.1016/0301-6226(93)90110-4

Stein, H. H., C. Pedersen, A. R. Wirt, and R. A. Bohlke. 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to pigs. J. Anim. Sci. 83:2387-2395. doi:10.2527/2005.83102387x

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. de Lange. 2007a. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282-285. doi:10.1016/j.livsci.2007.01.019

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

Stipanuk, M. H. 2013. Structure, nomenclature, and properties of proteins and amino acids. In: M. H. Stipanuk and M. A. Caudill, ed. Biochemical, Physiological, and Molecular Aspects of Human Nutrition. 3rd ed. Elsevier Saunders, St. Louis, MO. p. 69-90.

Volkert, D., and C. C. Sieber. 2011. Protein requirements in the elderly. Int. J. Vitam. Nutr. Res. 81:109-119. doi:10.1024/0300-9831/a000061

Williams, C. D. 1933. A nutritional disease of child-hood associated with a maize diet. Arch. Dis. Child. 8:423-433.

Yin, Y. -L., R. -L. Huang, and H. –Y Zhong. 1993. Comparison of ileorectal anastomosis and the conventional method for the measurement of ileal digestibility of protein sources and mixed diets in growing pigs. Anim. Feed. Sci. Tech. 42:297-308. doi:10.1016/0377-8401(93)90106-T

CHAPTER 3: VALUES FOR DIGESTIBLE INDISPENSABLE AMINO ACID SCORES (DIAAS) FOR SOME DAIRY AND PLANT PROTEINS MAY BETTER DESCRIBE PROTEIN QUALITY THAN VALUES CALCULATED USING THE CONCEPT FOR PROTEIN DIGESTIBILITY CORRECTED AMINO ACID SCORES (PDCAAS)

ABSTRACT: An experiment was conducted to compare values for digestible indispensable amino acid scores (DIAAS) for 4 animal proteins and 4 plant proteins with values calculated as recommended for protein digestibility corrected amino acid scores (PDCAAS), but determined in pigs instead of in rats. Values for standardized total tract digestibility (STTD) of crude protein (CP) and standardized ileal digestibility (SID) of amino acids were calculated for whey protein isolate (WPI), whey protein concentrate (WPC), milk protein concentrate (MPC), skim milk powder (SMP), pea protein concentrate (PPC), soy protein isolate (SPI), soy flour, and whole grain wheat. The PDCAAS-like values were calculated using the STTD of CP to estimate amino acid digestibility and values for DIAAS were calculated from values for SID of amino acids. Results indicated that values for SID of most indispensable amino acids in WPI, WPC, and MPC were greater ($P < 0.05$) than for SMP, PPC, SPI, soy flour, and wheat. With the exception of arginine and tryptophan, the SID of all indispensable amino acids in SPI was greater ($P < 0.05$) than in soy flour, and with the exception of threonine, the SID of all indispensable amino acids in wheat was less ($P < 0.05$) than in all other ingredients. If the same scoring pattern for children between 6 and 36 months was used to calculate PDCAAS-like values and DIAAS, PDCAAS-like values were greater ($P < 0.05$) than DIAAS values for SMP, PPC, SPI, soy flour, and wheat

indicating that PDCAAS-like values estimated in pigs may overestimate the quality of these proteins.

Key words: pigs, PDCAAS, DIAAS, protein quality, plant protein, dairy protein

INTRODUCTION

The protein digestibility corrected amino acid score (**PDCAAS**) has been used for more than 20 years to evaluate protein quality in human nutrition, but the PDCAAS procedure has limitations because values are calculated from the total tract digestibility of crude protein (**CP**) and calculations for PDCAAS are based on the assumption that all amino acids have the same digestibility as CP. It is, however, recognized that digestibility of amino acids is most correctly determined at the end of the small intestine (the ileum) because amino acids are absorbed only from the small intestine and because hindgut fermentation can affect fecal amino acid excretion (Sauer and Ozimek, 1986). Therefore, ileal digestibility is a more accurate estimate of amino acid bioavailability than total tract digestibility in both humans and pigs (Stein et al., 2007; Cervantes-Pahm et al., 2014). In addition, the digestibility of CP is not representative of the digestibility of all amino acids (Stein et al., 2007), because individual amino acids are digested with different efficiencies (Stein et al., 2007). Other criticisms of the PDCAAS procedure have been recently reviewed and include use of truncation to avoid having values greater than 1, use of a scoring pattern that is based on amino acid requirements for children, and use of metabolic fecal nitrogen to correct for endogenous losses of amino acids (Schaafsma, 2012; Gilani, 2012; Rutherfurd et al., 2015). It was also recently concluded that PDCAAS generally underestimates the value of high-quality proteins and overestimates the value of low-quality proteins (Rutherfurd et al., 2015).

To avoid the flaws of the PDCAAS procedure, the FAO now recommends an amino acid evaluation procedure called digestible indispensable amino acid score (**DIAAS**; FAO, 2013). To calculate DIAAS, it is necessary to determine the digestibility of individual amino acids at the end of the small intestine and the pig has been recognized as an appropriate model for estimating CP and amino acid digestibility in foods for humans (FAO, 2013; Rowan et al., 1994; Deglaire et al., 2009). In contrast, PDCAAS values according to the original definition are determined in rats (FAO, 1991). The apparent ileal digestibility of amino acids is defined as the net disappearance of ingested dietary amino acids from the digestive tract prior to the distal ileum (Stein et al., 2007). If values for apparent ileal digestibility are corrected for the basal endogenous losses of amino acids, the resulting values are described as standardized ileal digestibility (**SID**; Stein et al., 2007). Values for SID of amino acids are additive in mixed diets (Stein et al., 2005) and may be used to calculate DIAAS in proteins used in human nutrition (Cervantes-Pahm et al., 2014; (FAO, 2013).

Research in our laboratory estimated DIAAS in eight cereal grains by calculating SID values for all indispensable amino acids in pigs (Cervantes-Pahm et al., 2014). Results indicated that to meet dietary requirements for amino acids in humans, diets based on sorghum, wheat, rye, or maize require more amino acid supplementation than diets based on polished rice or de-hulled oats. However, in human nutrition, protein is usually supplied by either animal-based proteins or plant-based proteins. Animal proteins include a number of dairy products, and commonly used dairy proteins include whey protein concentrate (**WPC**), whey protein isolate (**WPI**), milk protein concentrate (**MPC**), and skim milk powder (**SMP**). Commonly used plant proteins include soy protein isolate (**SPI**), soy flour, and pea protein concentrate (**PPC**). To our knowledge, there are no published values for DIAAS for these proteins that have been

determined in pigs and it is not known how values for DIAAS determined in pigs compare with PDCAAS-like values determined in pigs. Therefore, the aim of this experiment was to compare PDCAAS-like values determined in pigs and values for DIAAS in 8 commonly used proteins and test the hypothesis that values for DIAAS are more appropriate to quantify protein quality than values for PDCAAS.

MATERIALS AND METHODS

Diets, Animals, Housing, and Experimental Design

The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. Four dairy proteins (WPI, WPC, MPC, and SMP) were procured from Cereal Byproducts Company, Mt. Prospect, Illinois, USA. Soy protein isolate and soy flour were obtained from Archer Daniels Midland Company, Decatur, Illinois, USA, and PPC was obtained from AGT Foods, Bismarck, North Dakota, USA. Wheat was obtained from Siemers, Teutopolis, Illinois, USA (Table 3.1). Each ingredient was included in one diet as the only source of CP and amino acids with the exception that wheat was included in combination with soy flour (Tables 3.2 and 3.3). A nitrogen-free diet was also formulated to measure basal endogenous losses of CP and amino acids. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates for growing pigs(NRC, 2012). All diets also contained 0.4% chromic oxide as an indigestible marker and all diets were provided in meal form.

Nine growing barrows (initial body weight: 26.25 ± 1.48 kg) were equipped with a T-cannula in the distal ileum using procedures adapted from Stein et al.(1998). Pigs were allowed a 7-d recovery after the surgery and they were then allotted to a 9 × 9 Latin square design with nine diets and nine 9-day periods. No pig received the same diet more than once during the

experiment and there was, therefore, nine replicate pigs per treatment. With nine replicates we expected to be able to detect differences in SID values among ingredients of 2.5 to 4 percentage units (depending on the amino acid). Pigs were housed in individual pens (0.9 × 1.8 m) in an environmentally controlled room. Pens had smooth sides and fully slatted concrete floors. A feeder and a nipple drinker were installed in each pen. At the conclusion of the experiment, pigs were approximately 19 weeks of age and had a body weight of 84.70 ± 6.48 kg.

Data Recording and Sample Collection

All pigs were fed their assigned diets in a daily amount of three times the estimated energy requirement for maintenance (824 kJ metabolizable energy per $kg^{0.60}$**;** NRC, 2012). The daily feed allotment was provided every day at 0800 h. Water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The amount of feed supplied each day was recorded as well. The initial five days of each period were considered an adaptation period to the diet. Fecal samples were collected on days 6 and 7 and immediately frozen at – 20°C. Ileal digesta were collected for 8 hours (from 0800 to 1600 h) on days 8 and 9 using standard operating procedures (Stein et al., 1998). Briefly, cannulas were opened and cleaned, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta or at least once every 30 min, and immediately frozen at – 20°C to prevent bacterial degradation of the amino acid in the digesta. Individual pig weights recorded at the conclusion of each period were used to calculate the feed provision for the subsequent period.

Chemical Analysis

At the conclusion of the experiment, fecal samples were dried in a forced air oven and finely ground through a 1-mm screen in a Wiley Mill (Model 4; Thomas Scientific, Swedesboro,

NJ) prior to analysis. Ileal samples were thawed, mixed within animal and diet, and a sub-sample was collected for analysis. A sample of each source of protein and of each diet was collected at the time of diet mixing. Digesta samples were lyophilized and finely ground prior to chemical analysis. Diets, ingredients, fecal samples, and ileal samples were analyzed for dry matter (Method 927.05; AOAC, 2007) and CP by combustion (Method 990.03; AOAC, 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Aspartic acid was used as a calibration standard and CP was calculated as N × 6.25. Samples were analyzed in duplicate, but analyses were repeated if the analyzed values were more than 5% apart. Diets, fecal samples, and ileal digesta were also analyzed in duplicate for chromium (Method 990.08; AOAC, 2007) and all diets, ingredients, and ileal digesta samples were analyzed for amino acids on a Hitachi Amino Acid Analyzer (Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for post-column derivatization and nor-leucine as the internal standard. Samples were hydrolyzed with 6*N* HCl for 24 h at 110°C prior to analysis, but methionine and cysteine were analyzed as methionine sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis and tryptophan was determined after NaOH hydrolysis for 22 h at 110°C [Method 982.30 E (a, b, c); (AOAC, 2007)].

Calculations

Values for apparent ileal digestibility of CP and amino acids, basal endogenous losses of CP and amino acids, and SID of CP and amino acids were calculated for all diets as previously explained (Stein et al., 2007). For all ingredients except wheat, the SID for CP and amino acids in the diets also represented the SID of the ingredient, but for wheat, the SID of CP and amino acids were calculated using the difference procedure (Rojas and Stein, 2013). Values for the

standardized total tract digestibility (STTD) of CP were calculated as explained for the calculation of SID of CP.

The concentration of SID amino acids (g/kg) in each ingredient was calculated by multiplying the SID value (%) for each amino acid by the concentration (g/kg) of that amino acid in the ingredient, and this value was then divided by the concentration of CP in the ingredient to calculate digestible indispensable amino acid content (mg) in 1 g protein (Cervantes-Pahm et al., 2014). The digestible indispensable amino acid reference ratios were calculated for each ingredient using the following equation (FAO, 2013): Digestible indispensable amino acid reference ratio = mg of digestible indispensable amino acid content in 1 g protein of food / mg of the same dietary indispensable amino acid in 1g of the reference protein. The reference proteins were based on FAO (FAO, 2013) and separate ratios were calculated using the reference protein for infants less than 6 months old, children from 6 months old to 36 months old, and children older than 36 months old, adolescents, and adults (FAO, 2013). The DIAAS values were then calculated using the following equations:

DIAAS (%) = 100 × lowest value of the digestible indispensable amino acid reference ratio (FAO, 2013).

Values for STTD of CP were used to calculate PDCAAS-like values using the following equation (Schaafsma, 2000):

PDCAAS-like values (%) = mg of limiting amino acid in 1g of test protein/mg of the same amino acid in 1 g of reference protein × standardized total tract digestibility (%) × 100.

Calculation of PDCAAS-like values used the reference protein for 2 to 5 year old children as recommended if values are calculated from STTD of CP in rats (FAO, 1991).

However, to allow for a direct comparison between PDCAAS-like values and values for DIAAS, PDCAAS-like values were also calculated using the three reference proteins that were used to calculate DIAAS values (FAO, 2013).

Statistical analyses

Normality of data was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Institute Inc., Cary, NC). Data were analyzed by ANOVA using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) in a randomized complete block design with the pig as the experimental unit. The statistical model to determine differences in SID of amino acid values among ingredients included diet as the main effect and pig and period as random effects. The model to compare values for SID and STTD of CP within each ingredient included calculation procedure (SID or STTD) as main effect and pig and period as random effects. The model to compare values for DIAAS and PDCAAS used calculation procedure (DIAAS or PDCAAS) as main effect and pig and period as random effects. Treatment means were calculated using the LSMEANS statement, and if significant, means were separated using the PDIFF option of the MIXED procedure. Significance and tendency was considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

All pigs remained healthy throughout the experiment and readily consumed their diets. Gross chemical composition of all ingredients was generally in agreement with published values (NRC, 2012). The concentration of CP in ingredients ranged from 11.67 to 92.66%.

With the exception of Tyr, the SID of all amino acids was not different between WPI and WPC (Table 3.4). The SID of Ile, Cys, and Ser was less ($P < 0.05$) in MPC than in WPI and WPC, and the SID of Val and Glu was less ($P < 0.05$) in MPC than in WPI, but for all other

amino acids, no differences among MPC, WPI, and WPC were observed. However, the SID of most amino acids was greater ($P < 0.05$) in WPI, WPC, and MPC than in SMP, PPC, soy flour, and wheat, but for SPI, many amino acids had SID values that were not different from those in WPI, WPC, and MPC. With the exception of Arg, Trp, Ala, and Gly, the SID of all amino acids was greater ($P < 0.05$) in SPI than in soy flour. The SID of Met, Trp, and Cys was less ($P < 0.05$) in PPC than in soy flour and the SID of Asp and Glu was greater ($P < 0.05$) in PPC than in soy flour, but for all other amino acids, no difference between these 2 ingredients was observed. The SID of all indispensable amino acids and of Ala and Tyr was less ($P < 0.05$) in wheat than in all other ingredients.

The SID of CP was greater ($P < 0.05$) than the STTD of CP for WPI, WPC, and wheat (Table 3.5). In contrast, the STTD of CP was greater ($P < 0.05$) than the SID of CP in MPC, SMP, and SPI, whereas no difference between SID and STTD of CP was observed for PPC and soy flour.

The protein digestibility corrected amino acid reference ratios calculated according to the recommendations from FAO/WHO (FAO, 1991) but using pigs instead of rats and based on the scoring pattern for preschool children (2 to 5 years old) are presented in Table 3.6. However, the protein digestibility corrected amino acid reference ratios calculated from STTD values of CP in pigs were also calculated according to FAO (FAO, 2013) and based on requirements of infants (birth to 6 months of age), children (6 months to 3 years of age), and older children (older than 3 years of age) adolescents, and adults, and these values are presented in Table 3.7. Likewise, the digestible indispensable amino acid reference ratios calculated according to FAO (FAO, 2013) and based on the same three age groups are presented in Table 3.8.

If PDCAAS-like values calculated according to FAO/WHO (FAO, 1991) were truncated as recommended, values for WPC, MPC, SMP were less ($P < 0.05$) than values for DIAAS, whereas PDCAAS-like values for PPC, SPI, soy flour, and wheat were greater ($P < 0.05$) than for DIAAS (Table 3.9). However, if PDCAAS-like values were not truncated, the PDCAAS-like value for WPC was not different from DIAAS, but PDCAAS-like values for MPC and SMP were greater ($P < 0.05$) than DIAAS. If PDCAAS-like values were calculated according to the same scoring pattern as DIAAS (FAO, 2013), PDCAAS-like values for SMP, PPC, SPI, soy flour, and wheat were greater ($P < 0.05$) than values for DIAAS, whereas the PDCAAS-like value for WPI was less ($P < 0.05$) than the DIAAS for WPI.

For DIAAS values, the first limiting amino acid in WPI and WPC was His, but for MPC, SMP, PPC, SPI, and soy flour, the sulfur amino acids were first limiting, and Lys was first liming in wheat. If PDCAAS-like values were calculated using the same scoring patterns as used to calculate DIAAS, the first limiting amino acid in the proteins was not different from those identified for DIAAS. However, if PDCAAS-like values were calculated using the original scoring patterns (FAO, 1991), either truncated or not truncated, the first limiting amino acid for whey proteins was the aromatic amino acids and Thr was first limiting in MPC and the sulfur amino acids were first limiting in SMP and SPI. However, the first limiting amino acid in PPC was Trp, whereas Lys was first limiting in soy flour and wheat.

Calculated PDCAAS-like values for infants were greater ($P < 0.05$) than values for DIAAS for SMP, PPC, SPI, and wheat, whereas the value for DIAAS for WPI tended ($P = 0.062$) to be greater than the PDCAAS-like value (Table 3.10). For children older than 3 years, adolescents, and adults, PDCAAS-like values for SMP, PPC, and SPI was greater ($P < 0.05$)

than DIAAS, and the PDCAAS-like value for soy flour tended ($P = 0.053$) to be greater than DIAAS. In contrast, the DIAAS for WPI was greater ($P < 0.05$) than the PDCAAS-like value.

The first limiting amino acids for DIAAS calculated for infants were the aromatic amino acids for the whey proteins, Trp for MPC and PPC, Thr for SMP, the sulfur amino acids for SPI, Leu for soy flour, and Lys for wheat. The first limiting amino acid for PDCAAS-like values calculated for infants in SMP was Trp, but for all other ingredients, the first limiting amino acid in the calculation of DIAAS was also first limiting for PDCAAS-like values. For children greater than 3 years old, adolescents and adults, the first limiting amino acid for both DIAAS and PDCAAS-like values for all proteins were the same as those identified for children from 6 months to 3 years old.

DISCUSSION

The amount and quality of protein consumed throughout the world varies depending on protein availability, amino acid composition of proteins, and digestibility of amino acids (Schaafsma, 2000). In many parts of the world, plant proteins are the primary sources of amino acids in the diet (Cervantes-Pahm et al., 2014; Schönfeldt and Hall, 2012; Swaminathan et al., 2012), whereas animal proteins are the primary sources of amino acids in other parts of the world (Swaminathan et al., 2012). However, the composition and digestibility of both of these types of proteins differ (Cervantes-Pahm et al., 2014; Gilani and Sepehr., 2003), and both plant and animal proteins, therefore, need to be evaluated. In the present experiment we attempted to do that, but it is acknowledged that all proteins were fed as raw ingredients without the processing that these ingredients most often go through before consumption by humans. If processing changes the digestibility of the protein, results may be different. Other limitations of the

experiment include the assumption that amino acid digestibility in growing castrated male pigs are representative of values obtained in both male and female humans of all ages.

In the current experiment, values for amino acid digestibility calculated from the total tract digestibility of CP were estimated from pigs although the rodent is the recommended model in the definition of PDCAAS (FAO, 1991). However, it was the objective to determine if total tract digestibility values for CP can be used to accurately estimate ileal digestibility values of individual amino acids and if we had used a rodent to calculate PDCAAS values and the pig to calculate DIAAS values, any differences would have been confounded by using the two different animal models. It is, therefore, important that the comparison is done within the same animal and because the pig has been recommended as the preferred animal model to calculate DIAAS values (FAO, 2013), we chose to use the pig to also calculate PDCAAS-like values in this study.

As expected, dairy proteins had greater SID values than the plant proteins and they are, therefore, considered high quality proteins for humans (James et al., 2014; McAllan et al., 2014; Stanstrup et al., 2014). Protein quality in WPC, SMP, and SPI or soy protein concentrate have been studied in rats, and results indicated that WPC had greater PDCAAS than SMP, SPI, and soy protein concentrate (Rutherfurd et al., 2015; Gilani and Sepehr., 2003). Results of this experiment agree with previous results and also indicate that the PDCAAS-like value for WPC is greater than for SMP and that the whey proteins have a more balanced amino acid profile compared with whole milk protein. The major protein in SMP is casein, which has a low concentration of cysteine, and this may be the reason for the reduced PDCAAS-like value for SMP compared with WPC.

According to the FAO recommended amino acid patterns for older children, adolescents, and adults and recommendations for nutrient claims, all dairy proteins tested in this experiment

can be considered “excellent/high” quality sources of protein, with DIAAS greater than or equal to 100 (FAO, 2013). By the same guidelines, SPI and soy flour qualify as “good” sources of protein, with a score greater than or equal to 75 and less than 100. In contrast, proteins with DIAAS less than 75 are recommended to make no claims regarding protein quality (FAO, 2013), and PPC and wheat tested in this experiment fall into this category. However, it is recognized that the cut-off values for protein quality assessments that were proposed were arbitrarily chosen and not based on documented research (FAO, 2013).

The nitrogen-free diet was used to estimate endogenous amino acid losses. Values obtained using this procedure are estimates for the basal endogenous losses that are independent of the diet and secreted only in response to dry matter being present in the small intestine (Stein et al., 2007). In addition to the basal endogenous losses, diet specific endogenous losses may also occur, but these losses will not be included in the values obtained from the nitrogen-free diet, and therefore, diet specific losses are debited against the ingredients in the calculations of SID values. Thus, if a specific diet or ingredient induces diet-specific endogenous losses because of high concentrations of dietary fiber or anti-nutritional factors, the SID values for that diet or ingredient will be reduced compared with values for a diet or ingredient that does not induce specific endogenous losses. However, because endogenous losses are truly lost from the body, values for SID will give a better estimate of the amino acids that are available for metabolism than if values for diet-specific endogenous losses had not been debited against the ingredient or diet. The calculated values for the SID of glycine in several ingredients exceeded 100% in the current experiment, which is not biologically possible, but these values are an artifact that is caused by an overestimation of endogenous glycine, which often happens when the nitrogen-free procedure is used to determine endogenous losses of amino acids (Stein et al., 2007).

For all proteins, SID values were different among both indispensable and dispensable amino acids indicating that one single value cannot be used to estimate the digestibility of individual amino acids as is assumed in the calculation of PDCAAS (FAO, 1991). For all ingredients used in this experiment with the exception of wheat, threonine had a lower SID value than lysine, which is usually the case for proteins that are not heat damaged. This is a result of the greater concentrations of threonine than of lysine and other indispensable amino acids in mucin protein secreted into the small intestine (Stein et al., 1999). Mucin protein is resistant to protease digestion, and therefore is included in the endogenous protein fraction that reaches the distal end of the ileum in pigs without being hydrolyzed. We are not aware of data for the amino acid composition of mucin in humans, but it has been reported that the ileal digestibility of threonine in humans is less than that of other indispensable amino acids, which indicates that mucin in humans also may have a high concentration of threonine (Rowan et al., 1994), (Deglaire et al., 2009). The observation that both lysine and tryptophan in wheat had a lower SID value than threonine may indicate that the wheat used in this experiment had been heat damaged during drying or grinding.

The differences between values for SID and STTD of CP that were observed are in agreement with reports indicating that the apparent ileal digestibility of CP is different from the apparent total tract digestibility of CP (Sauer and Ozimek, 1986; Knabe et al., 1989). In most cases, the total tract digestibility of CP is greater than the ileal digestibility because of absorption of ammonia from the hindgut (Hendriks et al., 2012; Boye et al., 2012), but as illustrated in this experiment, in some cases, nitrogen may be secreted into the hindgut resulting in a reduced value for STTD compared with SID. However, because nitrogen exchange in the hindgut does not contribute to the amino acid balance in humans and monogastric animals and because amino

acids are absorbed only in the small intestine, the differences between STTD and SID values illustrate why values for STTD do not always represent absorption of amino acids. Thus, the use of STTD of CP to estimate the digestibility of all amino acids in the PDCAAS system will result in inaccuracies of estimates for amino acid digestibility, which has also been previously illustrated (Rutherfurd et al., 2015; McAllan et al., 2014).

In addition to the lack of digestibility values for individual amino acids, a major limitation of the PDCAAS system is that all scores are truncated to 100% with the rationale that any amount of amino acids beyond the requirement pattern confers no additional benefit to the individual consuming the protein (FAO, 2013; Schaafsma, 2000; Boye et al., 2012; Sarwar, 1997). This assumption, however, neglects the complementary effect that excess amino acids may have in combination with amino acids from other proteins (Boye et al., 2012; Sarwar, 1997), and as a consequence, PDCAAS values do not give credit for extra indispensable amino acids that a protein may add to a diet (Boye et al., 2012; FAO, 2007). In contrast to the PDCAAS system, values for DIAAS are not truncated to 100%, and therefore, give credit to a protein based on its value as a complementary source of amino acids with other sources of proteins in a mixed diet (Rutherfurd et al., 2015).

Despite the challenges with the PDCAAS procedures, which have been previously reviewed (Schaafsma, 2012; Boye et al., 2012; Sarwar, 1997), it is important to recognize that criticism related to the scoring patterns that were originally suggested (FAO, 1991) can be easily overcome by adopting different scoring patterns. Indeed, in a later report from WHO/FAO, scoring patterns for several age groups of children, teenagers, and adults were suggested (FAO, 2007). Likewise, the problems associated with truncation can also be easily corrected by using un-truncated values (Boye et al., 2012). As a consequence, the principal methodological

difference between values calculated for PDCAAS and values calculated for DIAAS is related to the assumption that the small intestinal absorption of individual amino acids can be predicted from the total tract digestibility of CP. As was clearly illustrated in this experiment, differences in the ileal digestibility among individual amino acids in all proteins exist with the digestibility of threonine being the least for most proteins. As a consequence, the ileal digestibility of amino acids cannot be accurately predicted from a single value obtained for the total tract digestibility of CP. It is also clearly illustrated that both STTD and SID of CP overestimate the ileal digestibility of amino acids for proteins with lower amino acid digestibility and as a consequence, values for PDCAAS that are predicted from the STTD of CP are expected to be less accurate for proteins with low amino acid digestibility than for proteins with greater amino acid digestibility. These principles are illustrated by the data in Table 3.9 where PDCAAS-like values are calculated according to the original recommendation (FAO, 1991) with scoring patterns for 2 to 5 year old children and all values are truncated to 100. The observation that the PDCAAS-like values for WPC, MPC, and SMP are much less than values for DIAAS is a consequence of truncation. However, if values are not truncated, none of these proteins have PDCAAS-like values that are less than values for DIAAS. Indeed, removing the truncation resulted in PDCAAS-like values that were greater than values for DIAAS for 6 of the 8 protein sources, indicating an overestimation of protein quality by using PDCAAS-like values. Values for DIAAS were calculated based on the scoring pattern for children from 6 to 36 months (FAO, 2013), and because this scoring pattern is different from the original PDCAAS scoring pattern (FAO, 1991), this will influence the calculations. However, even if the PDCAAS-like values were calculated using the DIAAS scoring pattern, PDCAAS-like values for 5 of the 8 proteins were greater than values for DIAAS. This observation is a consequence of the fact that total tract

digestibility of CP is usually greater than the ileal digestibility of amino acids as discussed above, and as expected, the difference between PDCAAS-like values and DIAAS is greater for proteins with lower amino acid digestibility than for proteins with greater digestibility. Thus, it appears that the major inaccuracies in the calculation of PDCAAS are a consequence of the incorrect assumption that the ileal digestibility of all indispensable amino acids can be predicted from the total tract digestibility of CP. This inaccuracy will have greater impact on evaluation of proteins used in developing countries than in developed countries, because foods typically consumed in many developing countries have lower digestibility of CP than food typically consumed in developed countries (Gilani et al., 2005).

If PDCAAS-like values and DIAAS values were calculated for children older than 6 months or for adults and if the same scoring pattern was used, no differences between the 2 methodologies in terms of predicting the first limiting amino acid were observed with lysine being first limiting in wheat, histidine being first limiting in the whey proteins and the sulfur amino acids being first limiting in the whole milk proteins and the soy and pea proteins. However, if the original scoring pattern for PDCAAS was used, the predicted first limiting amino acids were different for all proteins except SMP, PPC, and wheat, which illustrates that the choice of scoring pattern will influence, which amino acid is predicted to be first limiting in a specific protein.

The observation that PDCAAS-like values and values for DIAAS were much less if the scoring pattern for infants (i.e., those under 6 months of age) was used instead of scoring patterns for older children or adults illustrate the high protein quality that is needed in proteins by infants. The fact that some of the proteins, such as PPC and wheat, have very low DIAAS and PDCAAS-

like values for infants is likely of minor consequence because these proteins are not expected to be used to a great extent in the feeding of infants.

CONCLUSION

In conclusion, data from this experiment indicate that PDCAAS-like values calculated from the total tract digestibility of CP in pigs and DIAAS values for dairy proteins are greater than for proteins obtained from soybeans, peas, or wheat. Data also indicate that for most proteins, significant differences between PDCAAS-like values and DIAAS were observed. Whereas some of the flaws in the calculation of PDCAAS can be corrected by using different scoring patterns, the fundamental problem with values for PDCAAS is that they are calculated using the incorrect assumption that the ileal digestibility of all amino acids can be predicted from the total tract digestibility of CP. Because of this assumption, PDCAAS values do not accurately predict ileal amino acid digestibility and it appears that specifically for low quality proteins, values for PDCAAS overestimate the protein quality. Thus, to better meet protein requirements of humans, specifically for individuals consuming diets that are low or marginal in digestible amino acids, values for DIAAS should be used to estimate protein quality of ingredients and diets.

TABLES

Table 3.1. Analyzed nutrient composition of ingredients (as-fed basis)[1]

	Ingredient[2]							
Item	WPI	WPC	MPC	SMP	PPC	SPI	Soy flour	Wheat
Dry matter, %	93.22	92.93	92.83	90.59	93.70	93.79	92.23	88.22
Crude protein, %	85.23	78.01	67.93	34.65	54.46	92.66	52.29	11.67
Calcium, %	0.36	0.36	1.77	1.15	0.08	0.05	0.28	0.04
Phosphorus, %	0.23	0.31	1.18	0.91	0.69	0.73	0.69	0.37
Indispensable amino acids, %								
Arg	1.96	2.38	2.45	1.20	4.83	6.95	3.71	0.56
His	1.71	1.72	2.04	1.07	1.43	2.41	1.43	0.30
Ile	5.95	4.94	3.61	1.80	2.31	4.38	2.35	0.39
Leu	9.91	9.27	6.91	3.47	4.04	7.38	4.00	0.78
Lys	8.64	7.83	5.50	2.90	4.11	5.69	3.30	0.39
Met	1.94	1.77	1.83	0.83	0.49	1.18	0.73	0.21
Phe	2.85	2.87	3.42	1.70	2.70	4.86	2.60	0.52

Table 3.1. (cont.)

Thr	6.58	5.39	3.02	1.50	1.95	3.35	2.00	0.34
Trp	1.83	1.57	1.01	0.54	0.48	1.30	0.79	0.12
Val	5.29	4.83	4.43	2.27	2.61	4.42	2.53	0.52
Dispensable amino acids, %								
Ala	4.58	4.20	2.27	1.14	2.25	3.74	2.20	0.44
Asp	10.22	8.79	5.29	2.68	5.99	10.56	5.84	0.62
Cys	2.14	1.91	0.46	0.26	0.63	1.06	0.72	0.25
Glu	15.97	13.62	14.55	7.37	8.62	17.10	9.20	3.06
Gly	1.57	1.62	1.31	0.68	2.25	3.77	2.16	0.50
Pro	5.35	4.50	6.69	3.33	2.17	4.65	2.52	1.03
Ser	4.10	3.86	3.51	1.81	2.37	4.25	2.33	0.49
Tyr	2.60	2.55	3.42	1.61	1.79	3.31	1.82	0.24

[1]The trypsin inhibitor units in soy flour and SPI were 8.06 and 2.75 units per mg, respectively.

[2]WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate.

Table 3.2. Ingredient composition of experimental diets (as-fed basis)[1]

	Diet[2]								
Ingredient, %	WPI	WPC	MPC	SMP	PPC	SPI	Soy flour	Wheat	N-free
WPI	21.00	-	-	-	-	-	-	-	-
WPC	-	23.00	-	-	-	-	-	-	-
MPC	-	-	40.00	-	-	-	-	-	-
SMP	-	-	-	50.00	-	-	-	-	-
PPC	-	-	-	-	25.00	-	-	-	-
SPI	-	-	-	-	-	21.00	-	-	-
Soy flour	-	-	-	-	-	-	35.00	11.30	-
Wheat	-	-	-	-	-	-	-	82.50	-
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	4.00
Solka floc	-	-	-	-	-	-	-	-	4.00
Monocalcium phosphate	1.60	1.60	1.60	1.60	1.60	1.60	1.60	0.80	2.40
Limestone	0.60	0.60	0.60	0.60	1.30	1.30	1.30	1.30	0.50
Sucrose	20.00	20.00	20.00	20.00	20.00	20.00	20.00	-	20.00
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Cornstarch	52.70	50.70	33.70	23.70	48.00	52.00	38.00	-	67.50
Magnesium oxide	-	-	-	-	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	-	-	-	-	0.40
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

Table 3.2. (cont.)

Vitamin micromineral premix[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1]All diets were formulated to contain approximately 17% crude protein, 0.70% calcium and 0.33% standardized total tract digestible phosphorus.

[2]WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate.

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 3.3. Analyzed nutrient composition of experimental diets (as-fed basis)

	Diet[1]								
Item	WPI	WPC	MPC	SMP	PPC	SPI	Soy flour	Wheat	N-Free
Dry matter, %	93.22	92.93	92.83	90.59	93.70	93.79	92.23	88.22	92.41
Crude protein, %	17.61	16.35	16.90	16.76	15.65	17.04	16.53	16.59	0.13
Indispensable amino acids, %									
Arg	0.39	0.49	0.58	0.55	1.23	1.27	1.13	1.00	0.01
His	0.38	0.41	0.52	0.51	0.41	0.49	0.48	0.46	0.02
Ile	1.27	1.08	0.91	0.88	0.64	0.86	0.77	0.69	0.01
Leu	2.09	2.07	1.71	1.65	1.10	1.42	1.28	1.22	0.02
Ly	1.85	1.72	1.37	1.38	1.13	1.12	1.05	0.80	0.02
Met	0.40	0.39	0.46	0.42	0.13	0.23	0.22	0.26	0.00
Phe	0.59	0.62	0.84	0.80	0.72	0.92	0.82	0.79	0.01
Thr	1.39	1.17	0.73	0.70	0.52	0.64	0.63	0.56	0.01
Tr	0.37	0.38	0.26	0.29	0.17	0.22	0.25	0.18	0.02
Val	1.15	1.05	1.13	1.08	0.72	0.89	0.82	0.80	0.01

Table 3.3. (cont.)

Total	9.88	9.38	8.51	8.26	6.77	8.06	7.45	6.76	0.13
Dispensable amino acids, %									
Ala	0.99	0.95	0.57	0.55	0.62	0.73	0.71	0.68	0.01
Asp	2.17	1.94	1.30	1.27	1.64	2.02	1.85	1.37	0.02
Cys	0.43	0.42	0.11	0.12	0.16	0.20	0.22	0.31	0.00
Glu	3.41	3.04	3.49	3.40	2.38	3.29	2.92	3.68	0.05
Gl	0.34	0.37	0.31	0.32	0.62	0.72	0.69	0.70	0.01
Ser	1.10	0.94	1.62	1.55	0.57	0.85	0.77	1.12	0.01
Tyr	0.94	0.86	0.83	0.79	0.62	0.79	0.70	0.69	0.01
Ala	0.46	0.48	0.74	0.70	0.43	0.54	0.54	0.51	0.01
Total	9.84	9.00	8.97	8.70	7.04	9.14	8.40	9.06	0.12
Total amino acids, %	19.72	18.38	17.48	16.96	13.81	17.20	15.85	15.82	0.25

[1]WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate.

Table 3.4. Standardized ileal digestibility of amino acids in ingredients[1]

	Ingredient[2]								Pooled SEM	*P*-value
Item	WPI	WPC	MPC	SMP	PPC	SPI	Soy flour	Wheat		
Indispensable amino acids, %										
Arg	104^{a}	101^{ab}	102^{ab}	98^{d}	99^{cd}	101^{bc}	99^{cd}	87^{e}	1.00	<0.05
His	100^{a}	97^{ab}	99^{a}	94^{bc}	95^{bc}	97^{ab}	92^{c}	85^{d}	1.55	<0.05
Ile	98^{a}	97^{ab}	93^{cd}	89^{e}	91^{d}	95^{bc}	92^{d}	86^{f}	1.00	<0.05
Leu	99^{a}	98^{a}	98^{a}	94^{b}	92^{c}	95^{b}	91^{c}	86^{d}	0.74	<0.05
Lys	98^{a}	96^{ab}	96^{ab}	95^{ab}	96^{ab}	97^{a}	93^{b}	77^{c}	1.31	<0.05
Met	98^{a}	97^{ab}	97^{ab}	96^{bc}	90^{e}	96^{c}	93^{d}	88^{f}	0.58	<0.05
Phe	98^{a}	96^{ab}	97^{a}	94^{b}	92^{c}	96^{ab}	92^{c}	87^{d}	0.82	<0.05
Thr	94^{a}	91^{abc}	93^{a}	82^{d}	88^{bc}	92^{ab}	87^{c}	80^{d}	1.91	<0.05
Trp	100^{a}	98^{ab}	97^{ab}	91^{d}	87^{e}	96^{bc}	92^{cd}	74^{f}	1.31	<0.05
Val	97^{a}	95^{ab}	94^{bc}	90^{d}	89^{d}	94^{b}	91^{cd}	83^{e}	1.22	<0.05
Mean	98^{a}	96^{a}	97^{a}	92^{b}	93^{b}	96^{a}	93^{b}	85^{c}	0.90	<0.05
Dispensable amino acids, %										

Table 3.4. (cont.)

Ala	98[a]	96[ab]	96[ab]	89[d]	92[cd]	96[abc]	93[bcd]	79[e]	1.51	<0.05
Asp	99[a]	96[ab]	97[ab]	88[c]	93[b]	95[ab]	88[c]	80[ab]	1.63	<0.05
Cys	98[a]	95[ab]	85[cd]	73[e]	75[e]	91[bc]	81[d]	86[cd]	2.57	<0.05
Glu	98[a]	96[abc]	94[bcd]	90[e]	96[ab]	97[a]	92[de]	93[cd]	1.19	<0.05
Gly	117[a]	112[a]	117[a]	96[b]	98[b]	100[b]	95[b]	87[c]	3.18	<0.05
Se	95[ab]	92[bc]	88[d]	80[e]	91[cd]	96[a]	92[bcd]	89[cd]	1.90	<0.05
Tyr	99[a]	96[bc]	98[ab]	95[cd]	93[d]	96[bc]	93[d]	90[e]	0.97	<0.05
Mean	102[a]	101[ab]	99[abc]	95[d]	98[bc]	101[ab]	96[cd]	94[d]	1.38	<0.05
Total amino acids	100[a]	98[a]	99[a]	94[b]	96[b]	99[a]	95[b]	90[c]	1.07	<0.05

[a-f]Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses of amino acids were calculated from pigs fed the nitrogen-free diet as follows (g/kg dry matter intake): arginine, 0.59; histidine, 0.20; isoleucine, 0.29; leucine, 0.49; lysine, 0.40; methionine, 0.08; phenylalanine, 0.29; threonine, 0.49; tryptophan, 0.10; valine, 0.40; alanine, 0.62; aspartic acid, 0.72; cysteine, 0.17; glutamic acid, 0.94; glycine, 1.50; serine, 0.43; tyrosine, 0.23.

[2]SEM = standard error of the mean; WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein

Table 3.4. (cont.)

concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate.

Table 3.5. Standardized ileal digestibility (SID) and standardized total tract digestibility (STTD) of crude protein (CP) in ingredients

	Ingredient[1]							
Item	WPI	WPC	MPC	SMP	PPC	SPI	Soy flour	Wheat
SID of CP, %	101	98	92	90	95	94	92	91
STTD of CP, %	96	97	97	96	94	96	90	86
SEM	2.7	0.9	3.5	3.6	1.8	0.6	3.1	4.5
P-value	0.003	0.025	0.008	0.001	0.208	<0.001	0.168	0.022

[1]SEM = standard error of the mean; WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate.

Table 3.6. Protein digestibility corrected amino acid (PDCAA) reference ratios for ingredients calculated according to requirement for 2 to 5 year old child[1]

	Ingredient[2]							
Item	WPI	WPC	MPC	SMP	PPC	SPI	Soy flour	Wheat
PDCAA reference ratio, 2 to 5years[3]								
His	1.03	1.13	1.53	1.56	1.29	1.32	1.30	1.17
Ile	2.43	2.20	1.84	1.78	1.41	1.63	1.45	1.03
Leu	1.72	1.75	1.49	1.46	1.05	1.16	1.05	0.87
Lys	1.71	1.68	1.35	1.38	1.21	1.02	0.98	0.50
Sulfur amino acids	1.87	1.84	1.31	1.21	0.77	0.93	1.00	1.36
Aromatic amino acids	0.99	1.07	1.55	1.45	1.22	1.35	1.21	0.89
Thr	2.22	1.98	1.27	1.22	0.98	1.02	1.02	0.74
Trp	1.91	1.78	1.31	1.36	0.75	1.23	1.24	0.81
Val	1.73	1.72	1.81	1.80	1.28	1.31	1.25	1.10

[1]Values for PDCAA were calculated from the total tract digestibility of crude protein in pigs.

Table 3.6. (cont.)

[2]WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate; AAA = aromatic amino acids (Phenylalanine + Tyrosine); SAA = sulfur amino acids (Methionine + Cysteine).

[3]PDCAA reference ratios were calculated using the recommended amino acid scoring pattern for preschool children (2 to 5 years). The indispensable amino acid reference patterns are expressed as mg amino acid /g protein: His, 19; Ile, 28; Leu, 66; Lys, 58; sulfur amino acids, 25; aromatic amino acids, 63; Thr, 34; Trp, 11; Val, 35 (FAO, 1991).

Table 3.7. Protein digestibility corrected amino acid (PDCAA) reference ratios for ingredients calculated according to requirements for infants (less than 6 months of age), child (6 months to 3 years) and older child (3 years and above)[1]

	Ingredient[2]							
Item	WPI	WPC	MPC	SMP	PPC	SPI	Soy flour	Wheat
PDCAA reference ratio, birth to 6 months[3]								
His	0.96	1.06	1.44	1.47	1.21	1.24	1.23	1.10
Ile	1.27	1.16	0.97	0.94	0.75	0.86	0.77	0.55
Leu	1.21	1.25	1.06	1.04	0.75	0.83	0.75	0.63
Lys	1.47	1.47	1.18	1.21	1.06	0.89	0.86	0.44
Sulfur amino acids	1.45	1.44	1.03	0.95	0.60	0.73	0.79	1.08
Aromatic amino acids	0.68	0.74	1.08	1.01	0.85	0.94	0.85	0.62
Thr	1.76	1.58	1.01	0.98	0.79	0.82	0.82	0.60
Trp	1.27	1.19	0.88	0.92	0.50	0.82	0.84	0.55
Val	1.13	1.13	1.19	1.19	0.84	0.87	0.83	0.73

Table 3.7. (cont.)

PDCAA reference ratio, 6 months to 3years[4]								
His	1.01	1.11	1.51	1.54	1.27	1.30	1.29	1.16
Ile	2.19	1.99	1.67	1.62	1.28	1.47	1.32	0.94
Leu	1.77	1.81	1.55	1.52	1.09	1.20	1.09	0.91
Lys	1.78	1.77	1.43	1.47	1.28	1.08	1.04	0.53
Sulfur amino acids	1.78	1.76	1.25	1.16	0.74	0.89	0.97	1.32
Aromatic amino acids	1.23	1.35	1.94	1.83	1.53	1.69	1.53	1.13
Thr	2.50	2.25	1.44	1.39	1.12	1.16	1.16	0.85
Trp	2.53	2.39	1.76	1.83	1.00	1.65	1.68	1.09
Val	1.45	1.45	1.52	1.52	1.08	1.11	1.06	0.93
PDCAA reference ratio, 3 years and above[5]								
His	1.26	1.39	1.88	1.93	1.59	1.62	1.61	1.45
Ile	2.33	2.13	1.78	1.73	1.37	1.57	1.41	1.00

Table 3.7. (cont.)

Leu	1.91	1.96	1.67	1.64	1.18	1.30	1.18	0.99
Lys	2.12	2.11	1.69	1.74	1.52	1.28	1.24	0.63
Sulfur amino acids	2.09	2.07	1.47	1.37	0.86	1.05	1.14	1.54
Aromatic amino acids	1.56	1.71	2.47	2.33	1.94	2.15	1.94	1.43
Thr	3.09	2.78	1.79	1.73	1.38	1.44	1.44	1.05
Trp	3.26	3.07	2.26	2.36	1.29	2.12	2.16	1.40
Val	1.55	1.56	1.64	1.64	1.16	1.19	1.14	1.00

[1]Values for PDCAA were calculated from the total tract digestibility of crude protein in pigs.

[2]WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate; AAA = aromatic amino acids (phenylalanine + tyrosine); SAA = sulfur amino acids (methionine + cysteine).

[3]PDCAA reference ratios were calculated using the recommended amino acid scoring pattern for an infant (birth to 6 months). The indispensable amino acid reference patterns are expressed as mg amino acid /g protein: His, 21; Ile, 55; Leu, 96; Lys, 69; sulfur amino acids, 33; aromatic amino acids, 94; Thr, 44; Trp, 17; Val, 55 (FAO, 2013).

[4]PDCAA reference ratios were calculated using the recommended amino acid scoring pattern for a child (6 months to 3 years). The

Table 3.7. (cont.)

indispensable AA reference patterns are expressed as mg amino acid /g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; sulfur amino acids, 27; aromatic amino acids, 52; Thr, 31; Trp, 8.5; Val, 40 (FAO, 2013).

[5]PDCAA reference ratios were calculated using the recommended amino acid scoring pattern for older child, adolescent, and adult. The indispensable AA reference patterns are expressed as mg amino acid /g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; sulfur amino acids, 23; aromatic amino acids, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

Table 3.8. Digestible indispensable amino acid (DIAA) reference ratios for ingredients calculated according to requirements for infants (less than 6 months of age), child (6 months to 3 years) and older child (3 years and above)[1]

	Ingredient[2]							
Item	WPI	WPC	MPC	SMP	PPC	SPI	Soy flour	Wheat
DIAA reference ratio, birth to 6 months[3]								
His	0.95	1.02	1.41	1.38	1.18	1.20	1.20	1.04
Ile	1.25	1.11	0.90	0.84	0.70	0.82	0.75	0.52
Leu	1.20	1.21	1.04	0.98	0.71	0.79	0.73	0.60
Lys	1.44	1.43	1.12	1.15	1.05	0.87	0.85	0.37
Sulfur amino acids	1.42	1.37	0.97	0.86	0.51	0.68	0.73	1.04
Aromatic amino acids	0.67	0.71	1.05	0.96	0.81	0.90	0.83	0.60
Thr	1.65	1.43	0.94	0.81	0.72	0.76	0.76	0.53
Trp	1.26	1.18	0.85	0.83	0.45	0.79	0.82	0.45
Val	1.09	1.07	1.11	1.07	0.78	0.82	0.80	0.67

Table 3.8. (cont.)

DIAA reference ratio, 6 months to 3 years[4]								
His	1.00	1.07	1.48	1.45	1.24	1.26	1.26	1.09
Ile	2.15	1.91	1.55	1.44	1.21	1.41	1.29	0.89
Leu	1.75	1.76	1.52	1.43	1.03	1.15	1.06	0.88
Lys	1.75	1.73	1.36	1.40	1.27	1.05	1.03	0.45
Sulfur amino acids	1.74	1.68	1.18	1.05	0.62	0.84	0.89	1.27
Aromatic amino acids	1.21	1.28	1.89	1.74	1.46	1.63	1.50	1.09
Thr	2.34	2.03	1.34	1.14	1.02	1.08	1.07	0.75
Trp	2.52	2.36	1.70	1.66	0.90	1.58	1.64	0.90
Val	1.40	1.36	1.43	1.37	0.99	1.05	1.03	0.85
DIAA reference ratio, 3 years and above[5]								
His	1.25	1.33	1.85	1.81	1.55	1.57	1.57	1.37
Ile	2.29	2.04	1.65	1.54	1.29	1.50	1.38	0.95

Table 3.8. (cont.)

Leu	1.89	1.90	1.64	1.55	1.12	1.24	1.14	0.95
Lys	2.08	2.06	1.61	1.66	1.50	1.25	1.23	0.53
Sulfur amino acids	2.04	1.97	1.41	1.24	0.73	0.98	1.05	1.49
Aromatic amino acids	1.53	1.63	2.40	2.21	1.85	2.06	1.90	1.38
Thr	2.90	2.52	1.66	1.42	1.26	1.33	1.33	0.93
Trp	3.24	3.04	2.19	2.14	1.16	2.04	2.12	1.16
Val	1.50	1.47	1.53	1.47	1.07	1.12	1.10	0.92

[1]Values for PDCAA were calculated from the total tract digestibility of CP in pigs.

[2]WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate; AAA = aromatic amino acids (phenylalanine + tyrosine); SAA = sulfur amino acids (methionine + cysteine).

[3]DIAA reference ratios and respective DIAAS were calculated using the recommended amino acid scoring pattern for an infant (birth to 6 months). The indispensable amino acid reference patterns are expressed as mg amino acid /g protein: His, 21; Ile, 55; Leu, 96; Lys, 69; sulfur amino acid, 33; aromatic amino acid, 94; Thr, 44; Trp, 17; Val, 55 (FAO, 2013).

[4]DIAA reference ratios and respective DIAAS were calculated using the recommended amino acid scoring pattern for a child (6 months to 3 years). The indispensable AA reference patterns are expressed as mg amino acid /g protein: His, 20; Ile, 32; Leu, 66; Lys,

Table 3.8. (cont.)

57; sulfur amino acid, 27; aromatic amino acid, 52; Thr, 31; Trp, 8.5; Val, 40 (FAO, 2013).

[5]DIAA reference ratios and respective DIAAS were calculated using the recommended amino acid scoring pattern for older child, adolescent, and adult. The indispensable AA reference patterns are expressed as mg amino acid /g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; sulfur amino acid, 23; aromatic amino acid, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

Table 3.9. Comparison of protein digestibility corrected amino acid scores (PDCAAS) and digestible indispensable amino acid scores (DIAAS) based on different requirement patterns[1]

Ingredient[2]	PDCAAS 1991[3]	PDCAAS 1991, untruncated	PDCAAS 2013[4]	DIAAS	SEM	*P*-value
WPI	99[a] (AAA)	99[b] (AAA)	97[c] (His)	100[a] (His)	0.3	<0.05
WPC	100[b] (AAA)	107[a] (AAA)	107[a] (His)	107[a] (His)	0.4	<0.05
MPC	100[c] (Thr)	127[a] (Thr)	121[b] (SAA)	120[b] (SAA)	0.5	<0.05
SMP	100[d] (SAA)	121[a] (SAA)	112[b] (SAA)	105[c] (SAA)	1.1	<0.05
PPC	75[a] (Trp)	75[a] (Trp)	71[b] (SAA)	62[c] (SAA)	0.6	<0.05
SPI	93[a] (SAA)	93[a] (SAA)	86[b] (SAA)	84[c] (SAA)	0.5	<0.05
Soy flour	98[a] (Lys)	98[a] (Lys)	93[b] (SAA)	89[c] (SAA)	1.3	<0.05
Wheat	50[a] (Lys)	50[a] (Lys)	51[a] (Lys)	45[b] (Lys)	1.3	<0.05

[a,b,c,d] Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Values for PDCAAS were calculated from the total tract digestibility of crude protein in pigs and values for DIAAS were calculated from the ileal digestibility of amino acids in pigs. First limiting amino acid is in parenthesis.

[2]SEM = standard error of the mean; WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate; AAA = aromatic amino acids

Table 3.9.

(phenylalanine + tyrosine); SAA = sulfur amino acids (methionine + cysteine).

[3]PDCAAS were calculated using the recommended amino acid scoring pattern for preschool children (2 to 5 years). The indispensable amino acids reference patterns are expressed as mg amino acid/g protein: histidine, 19; isoleucine, 28; leucine, 66; lysine, 58; sulfur amino acids, 25; aromatic amino acids, 63; threonine, 34; tryptophan, 11; valine, 35 (FAO, 1991).

[4]PDCAAS and DIAAS were calculated using the recommended amino acid scoring pattern for a child (6 months to 3years). The indispensable amino acid reference patterns are expressed as mg amino acid/g protein: histidine, 20; isoleucine, 32; leucine, 66; lysine, 57; sulfur amino acids, 27; aromatic amino acids, 52; threonine, 31; tryptophan, 8.5; valine, 40 (FAO, 2013).

Table 3.10. Comparison of protein digestibility corrected amino acid scores (PDCAAS) and digestible indispensable amino acid scores (DIAAS)[1]

	Ingredients[2]							
Item	WPI	WPC	MPC	SMP	PPC	SPI	Soy flour	Wheat
birth to 6 months[3]								
DIAAS	67 (AAA[4])	71 (AAA)	85 (Trp)	81 (Thr)	45 (Trp)	68 (SAA)	73 (Leu)	37 (Lys)
PDCAAS	66 (AAA)	72 (AAA)	85 (Trp)	88 (Trp)	49 (Trp)	71 (SAA)	72 (Leu)	42 (Lys)
SEM	0.30	0.48	0.51	2.4	0.42	0.68	0.83	1.2
P-value	0.062	0.164	0.743	0.039	<0.0001	0.026	0.642	0.017
3 years and above[4]								
DIAAS	125 (His)	133 (His)	141 (SAA)	123 (SAA)	73 (SAA)	98 (SAA)	105 (SAA)	54 (Lys)
PDCAAS	122 (His)	134 (His)	142 (SAA)	132 (SAA)	84 (SAA)	102 (SAA)	109 (SAA)	51 (Lys)
SEM	0.44	0.68	0.73	1.6	0.62	0.98	1.4	1.7
P-value	<0.001	0.311	0.196	0.002	<0.0001	0.028	0.053	0.220

[a,b,c,d] Means within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Values for PDCAAS were calculated from the total tract digestibility of crude protein in pigs and values for DIAAS were calculated from the ileal digestibility of amino acids in pigs. First limiting amino acid is in parenthesis.

Table 3.10. (cont.)

[2]SEM = standard error of the mean; WPI = whey protein isolate; WPC = whey protein concentrate; MPC = milk protein concentrate; SMP = skim milk powder; PPC = pea protein concentrate; SPI = soy protein isolate; AAA = aromatic amino acids (phenylalanine + tyrosine); SAA = sulfur amino acids (methionine + cysteine).

[3]PDCAAS and DIAAS were calculated using the recommended amino acid scoring pattern for an infant (birth to 6 months). The indispensable amino acid reference patterns are expressed as mg amino acid/g protein: histidine, 21; isoleucine, 55; leucine, 96; lysine, 69; sulfur amino acids, 33; aromatic amino acids, 94; threonine, 44; tryptophan, 17; valine, 55 (FAO, 2013).

[4]PDCAAS and DIAAS were calculated using the recommended amino acid scoring pattern for children older than 3 years, adolescents, and adults. The indispensable amino acid reference patterns are expressed as mg amino acid/g protein: histidine, 16; isoleucine, 30; leucine, 61; lysine, 48; sulfur amino acids, 23; aromatic amino acids, 41; threonine, 25; tryptophan, 6.6; valine, 40 (FAO, 2013).

LITERATURE CITED

AOAC Int. 2007. Official Methods of Analysis. 18th ed. Rev. 2. W. Howitz, and G. W. Latimer Jr., AOAC Int., Gaithersburg, MD.

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

Cervantes-Pahm, S. K., Y. Liu, and H. H. Stein. 2014. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br. J. Nutr. 111:1663-1672. doi:10.1017/s0007114513004273

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

FAO. 1991. Protein quality evaluation. Report of a joint FAO/WHO expert consultation. FAO food and nutrition paper 51. Food and Agriculture Organization of the United Nations. Bethesda, 1991.

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. FAO food and nutrition paper 92. Food and Agriculture Organization of the United Nations. Rome, 2013.

Gilani, G. S. 2012. Background on international activities on protein quality assessment of foods. Br. J. Nutr. 108:S168-S182. doi:10.1017/S0007114512002383

Gilani G. S., K. A. Cockell, and E. Sepehr. 2005. Effects of antinutritional factors on protein digestibility and amino acid availability in foods. J. AOAC Intl. 88:967-987.

Gilani G. S., and E. Sepehr. 2003. Protein digestibility and quality in products containing antinutritional factors are adversely affected by old age in rats. J. Nutr. 133:220-225. doi:10.1093/jn/133.1.220

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

James L.J., L. Mattin, P. Aldiss, R. Adebishi, and R. M. Hobson. 2014. Effect of whey protein isolate on rehydration after exercise. Amino Acids 46:1217-1224. doi:10.1007/s00726-014-1680-8

Knabe D.A., D. C. LaRue, E. J. Gregg, G. M. Martinez, and T. D. Tanksley Jr. 1989. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J. Anim. Sci. 67:441-458. doi:10.2527/jas1989.672441x

McAllan L., P. Skuse, P. D. Cotter, P. O'Connor. J. F. Cryan, R. P. Ross, G. Fitzgerald, H. M. Roche, and K. N. Nilaweera. 2014. Protein quality and the protein to carbohydrate ratio within a high fat diet influences energy balance and the gut microbiota in C57BL/6J mice. PLoS One 2:1-13. doi:10.1371/journal.pone.0088904

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC. doi:10.17226/13298

Rojas, O. J. and H. H. Stein. 2013. Concentration of digestible and metabolizable energy and digestibility of amino acids in chicken meal, poultry byproduct meal, hydrolyzed porcine intestines, a spent hen-soybean meal mixture, and conventional soybean meal fed to weanling pigs. J. Anim. Sci. 91:3220-3230. doi:10.2527/jas.2013-6269

Rowan A.M., P. J. Moughan, M. N. Wilson, K. Maher, and C. Tasman-Jones. 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: 29-42. doi:10.1079/BJN19940108

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

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. 127:758-764. doi:10.1093/jn/127.5.758

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

Schaafsma, G. 2000. The protein digestibility-corrected amino acid score. J. Nutr. 130:1865S-1867S. doi:10.1093/jn/130.7.1865S

Schaafsma, G. 2012. Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. Br. J. Nutr. 108:S333-S336. doi:10.1017/S0007114512002541

Schönfeldt H. C., and N. Hall. 2012. Dietary protein quality and malnutrition in Africa. Br. J. Nutr. 108:S69-S76. doi:10.1017/S0007114512002553

Stanstrup J., S. S. Schou, J. Holmer-Jensen, K. Hermansen, and L. O. Dragsted. 2014. Whey protein delays gastric emptying and suppresses plasma fatty acids and their metabolites

compared to casein, gluten and fish protein. J. Proteome Res. 13:2396-2408. doi:10.1021/pr401214w

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. de Lange. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282-285. doi:10.1016/j.livsci.2007.01.019

Stein, H. H. , C. Pedersen, A. R. Wirt, R. A. Bohlke. 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J. Anim. Sci. 83:2387-2395. doi:10.2527/2005.83102387x

Stein, H. H., C. F. Shipley, and R. A. Easter. 1998. Technical note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1443-1436. doi:10.2527/1998.7651433x

Stein H. H., N. L. Trottier, C. Bellaver, R. A. Easter. 1999. The effect of feeding level and physiological status on total flow and amino acid composition of endogenous protein at the distal ileum in swine. J. Anim. Sci. 77:1180-1187. doi:10.2527/1999.7751180x

Swaminathan S., M. Vaz, and A. V. Kurpad. 2012. Protein intakes in India. Br. J. Nutr. 108: S50-S68. doi:10.1017/S0007114512002413

WHO. 2007. Protein and amino acid requirements in human nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation. WHO Technical Report Series 935. World Health Organization. Geneva, 2007.

CHAPTER 4: PREPARATION OF PORK LOIN MAY INCREASE DIGESTIBLE INDISPENSABLE AMINO ACID SCORES WHEN DETERMINED USING PIGS

ABSTRACT: An experiment was conducted to characterize the amino acid concentration and digestible indispensable amino acid scores (DIAAS) of raw pork loin and to determine the effect of different preparations (i.e., roasting, frying, or grilling) on amino acid concentrations and DIAAS in pork loin. The DIAAS were calculated based on ileal digestibility of amino acids in pigs for raw pork loin, roasted pork loin, grilled pork loin, fried pork loin, and casein. Six ileal-cannulated barrows were allotted to a 6 × 6 Latin square design with 6 diets and 6 periods during which ileal effluent samples were collected to determine amino acid digestibility. A N-free diet was formulated to determine basal endogenous losses of amino acids and crude protein (CP) and to enable the calculation of standardized ileal digestibility (SID) of amino acids. The remaining diets were formulated with each test ingredient as the sole source of amino acids. Using determined values for SID of amino acids for each ingredient and established reference protein patterns, DIAAS were calculated. For children from birth to 6 m, fried pork loin had the greatest ($P < 0.05$) DIAAS followed by grilled pork loin, roasted pork loin, raw pork loin, and casein. For children from 6 m to 3 y, DIAAS were greatest ($P < 0.05$) for grilled and fried pork loin and least ($P < 0.05$) for raw pork loin and the DIAAS of roasted pork loin was greater ($P < 0.05$) than that of casein. For DIAAS calculated for children older than 3 y, there were no differences in the DIAAS among grilled pork loin, fried pork loin, and casein, but these 3 ingredients had greater ($P < 0.05$) DIAAS than roasted pork loin, which in turn had a greater ($P < 0.05$) DIAAS than raw pork loin. Results indicate that prepared pork loins can be considered excellent protein

sources based on their DIAAS and these data make it possible to calculate DIAAS for meals containing commonly consumed pork loin products. Additionally, results of this research indicate that even for high-quality proteins, such as pork loin, correct preparation can improve DIAAS.

INTRODUCTION

The Protein Digestibility Corrected Amino Acid Score (**PDCAAS**) has been used for more than 25 years to evaluate protein quality in human nutrition (FAO, 1991; WHO, 2007). However, this method has limitations because it is based on the total tract digestibility of CP and it is assumed that all amino acids have the same digestibility as CP. It is, however, recognized that digestibility of amino acids is most correctly determined at the end of the small intestine (the "ileum") because amino acids are absorbed only from the small intestine and because hindgut fermentation can affect fecal amino acid excretion (Sauer and Ozimek, 1986). Therefore, ileal digestibility is a more accurate estimate of amino acid bioavailability than total tract digestibility in both humans and pigs (Stein et al., 2007; Cervantes-Pahm et al., 2014; Mathai et al., 2017). In addition, the digestibility of CP is not representative of the digestibility of all amino acids (Stein et al., 2007; Mathai et al., 2017). Instead, individual digestibility values for each amino acid need to be used. As a consequence, the Food and Agriculture Organization of the United Nations (**FAO**) recommends a new amino acid evaluation procedure called "Digestible Indispensable Amino Acid score (**DIAAS**)". To calculate DIAAS, it is necessary that ileal amino acid digestibility values are generated and the pig has been recognized as the best animal model for estimating amino acid digestibility in humans using ileal digestibility (Rowan et al., 1994; Deglaire et al., 2009; FAO, 2013). The FAO also recommends that DIAAS be generated for all

proteins used in human nutrition, which will enable nutritionists to determine the quality of protein in meals fed to humans, thus ensuring deficiencies of amino acids are avoided. However, there is a lack of information about DIAAS of proteins used in human nutrition. Research in our laboratory has determined DIAAS in eight cereal grains (Cervantes-Pahm et al., 2014) and in several dairy and plant proteins (Mathai et al., 2017; Abelilla et al., 2018). Results of this research clearly demonstrate that cereal grains do not provide digestible amino acids in quantities that meet the requirement for amino acids by children. It is, therefore, necessary that additional sources of amino acids are provided, and pork protein is a readily available source of protein in many countries in the world. In the recent FAO report (FAO, 2013), it is stated that DIAAS for all food proteins need to be generated and specifically, there is a need to generate DIAAS for meat products. Therefore, research characterizing DIAAS in raw and prepared pork loin fills a void in our understanding of the nutritional value of proteins used in foods. Roasting, frying, or grilling are common ways to prepare pork loin, but limited research has been conducted to determine the effects of these preparation methods on the digestibility of amino acids. Therefore, it was the objective of this research to test the hypothesis that roasting, frying, or grilling will increase DIAAS in pork loin.

MATERIALS AND METHODS

Diets, Animals, Housing, and Experimental Design

All animal care procedures were conducted under a research protocol approved by the Institutional Animal Care and Use Committee, University of Illinois, Urbana. Pork loins were sourced from commercial sources by the University of Illinois Meat Science Laboratory and divided into 4 batches (Table 4.1). Pork loins were from pigs fed a common industry diet so the composition of the test loins were representative of loins purchased by consumers. One batch

was ground, vacuum packaged, and then frozen to provide raw pork loins, whereas the remaining 3 batches were roasted, grilled, or fried. Pork loins were either roasted using a commercial smokehouse (Middleby Corporation, Alkar Smoker, Elgin, IL) fried using a griddle and a small amount of vegetable oil to simulate in-home fried pork chops, or grilled (Farberware® OPEN HEARTH® Grill, Garden City, NY). For all cooking methods, pork loins were cooked to an internal temperature of 145°F, which is the United States Department of Agriculture recommended temperature to ensure food safety, and following preparation, loins were ground, vacuum packaged, and frozen until use.

Four diets were based on each of the 4 batches of pork loin (i.e., raw, roasted, fried, or grilled) as the sole source of amino acids. A control diet was also formulated containing casein as the sole proteinaceous ingredient. All diets contained 10% CP on a dry matter (**DM**) basis (Tables 4.2 and 4.3). The last diet was a N-free diet that was used to estimate basal endogenous losses of CP and amino acids, which was necessary for the calculation of DIAAS. The N-free and casein diets were fed as-is. However, pork ingredients were combined daily with sufficient N-free diet to provide 10% CP on a DM basis. Cornstarch, sucrose, soybean oil, vitamins, and minerals were also included in the diets to ensure that all nutrient requirements of growing pigs were met (NRC, 2012). All diets were provided in meal form and titanium dioxide was used as an indigestible marker.

Six gilts (initial BW: 26.16 ± 4.68 kg) were surgically fitted with a T-cannula in the distal ileum (Stein et al., 1998). After surgery, pigs were housed individually in pens (2 x 3m) that were equipped with a feeder and a nipple drinker and concrete half-slatted floor. Pigs were then allotted to a 6 × 6 Latin square design with 6 diets and 6 periods comprising the rows and columns of the square. Pigs were fed their respective diets in quantities equivalent to 4% of their

total BW. The daily feed allotment was divided into 2 meals that were provided every day at 0800 and 1600 h. Water was available at all times and each period lasted 7 d.

Data Recording and Sample Collection

Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The amount of feed supplied each day was recorded, as well. The initial 5 days of each period were considered an adaptation period to the diet. Ileal digesta were collected for 8 h (from 0800 to 1600 h) on d 6 and 7 using standard operating procedures (Stein et al., 1998). Briefly, cannulas were opened and cleaned, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta or at least once every 30 min, and immediately frozen at – 20^{o}C to prevent bacterial degradation of the amino acids in the digesta. Individual pig weights recorded at the conclusion of each period were used to calculate the feed provision for the subsequent period.

Chemical Analysis

Ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized and ground through a 1-mm screen in a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ). Diets and all ileal digesta samples were analyzed for dry matter (DM; Method 927.05; AOAC International, 2007) and CP (Method 990.03; AOAC International, 2007) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI) at the Monogastric Nutrition Laboratory at the University of Illinois. Pork loin samples were analyzed for CP by measuring N concentration using the Kjeldahl method (method 976.05; AOAC Int., 2007) due to their high moisture contents. Samples were analyzed in duplicate, but analyses were repeated if the analyzed values were more than 5% apart. All samples were also analyzed for amino acids

[Method 982.30 E (a, b, c); AOAC International, 2007], and samples of diets and ileal digesta were analyzed for Ti (Myers et al., 2004).

Calculations

Apparent ileal digestibility values for amino acids in the protein sources were calculated using equation [1] (Stein et al., 2007):

$$AID\ (\%) = [1 - [(AAd/AAf) \times (Tif/Tid)] \times 100 \quad \textbf{[1]}$$

where AID is the apparent ileal digestibility of an amino acid (%), AAd is the concentration of that amino acid in the ileal digesta DM, AAf is the amino acid concentration of that amino acid in the feed DM, Tif is the titanium concentration in the feed DM, and Tid is the titanium concentration in the ileal digesta DM.

The basal endogenous flow to the distal ileum of each amino acid was determined based on the flow obtained after feeding the N-free diet using equation [2] (Stein et al., 2007):

$$IAA_{end} = [AAd \times (Tif/Tid)] \quad \textbf{[2]}$$

where IAA_{end} is the basal endogenous loss of an amino acid (mg per kg DM intake). The basal endogenous loss of CP was determined using the same equation.

By correcting the AID for the IAA_{end} of each amino acid, standardized ileal amino acid digestibility values were calculated using equation [3] (Stein et al., 2007):

$$SID = [(AID + IAA_{end})/AAf] \quad \textbf{[3]}$$

where SID is the standardized ileal digestibility value (%).

The concentration of SID amino acids (g/kg) in each ingredient was calculated by multiplying the SID value (%) for each amino acid by the concentration (g/kg) of that amino acid in the ingredient, and this value was then divided by the concentration of CP in the ingredient to calculate the quantity of digestible indispensable amino acid (mg) in 1 g protein (Cervantes-

Pahm et al., 2014; Mathai et al., 2017). The digestible indispensable amino acid reference ratios were calculated for each ingredient using the following equation [4] (FAO, 2013):

Digestible indispensable amino acid reference ratio = digestible indispensable amino acid content in 1 g protein of food (mg) / mg of the same dietary indispensable amino acid in 1g of reference protein. **[4]**

The reference proteins were based on FAO (2013) definitions and separate ratios were calculated using the reference protein for infants less than 6 months old, children from 6 months old to 36 months old, and children older than 36 months old, adolescents, and adults. The DIAAS were then calculated using the following equation [5] (FAO, 2013):

DIAAS (%) = 100 × lowest value of digestible indispensable amino acid reference ratio. **[5]**

Statistical Analyses

Normality of data was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Inst. Inc., Cary, NC). Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the pig as the experimental unit. The statistical model to determine differences in SID of amino acid values among ingredients included diet as the main effect and pig and period as random effects. Treatment means were calculated using the LSMEANS statement, and when significantly different, means were separated using the PDIFF option of the MIXED procedure. Significance was considered at $P < 0.05$.

RESULTS

All pigs remained healthy throughout the experiment and readily consumed their diets. For indispensable amino acids, the AID and SID of Ile and Thr were less ($P < 0.05$) in casein than in any of the pork products (Tables 4.4 and 4.5). The AID and SID of His, Ile, Leu, Lys, Met, Phe, Thr, and Val were less ($P < 0.05$) in fried pork loin when compared with the other pork products. The SID of His and Thr were greater ($P < 0.05$) in raw pork loin than in any of the other ingredients.

For DIAAS calculated for children from birth to 6 m, fried pork loin had the greatest ($P < 0.05$) values followed by grilled pork loin, roasted pork loin, raw pork loin, and casein (Table 4.6). For DIAAS calculated for children from 6 m to 3 y, values were greatest ($P < 0.05$) for grilled pork loin and fried pork loin and least ($P < 0.05$) for raw pork loin. The DIAAS of roasted pork loin was greater ($P < 0.05$) than that of casein. For DIAAS calculated for children older than 3 y, there were no differences between grilled pork loin, fried pork loin, or casein, but these 3 ingredients had greater ($P < 0.05$) DIAAS than roasted pork loin, which in turn had a greater ($P < 0.05$) DIAAS than raw pork loin.

For DIAAS calculated for infants, the first limiting amino acid in raw pork loin was His, whereas for all other ingredients the first limiting amino acid was Trp. However, for children from 6 m to 3 y, His was the first-limiting amino acid in all pork products and Trp was the first-limiting amino acid in casein. For children older than 3 y, His was the first limiting amino acid in raw pork loin, Leu was the first limiting amino acid in fried, grilled, or roasted pork loin, and Trp was the first-limiting amino acid in casein.

DISCUSSION

Due to the importance of amino acids nutrition in humans, it is imperative that knowledge about amino acid digestibility is generated because that will allow formulation of diets that can alleviate amino acid malnutrition specifically in vulnerable groups such as children and lactating women (WHO, 2007; Pillai and Kurpad, 2012; FAO, 2013). To accomplish this, it is necessary to prepare meals that provide all amino acids in the required quantities, but that is not possible unless values for DIAAS of the individual food proteins are known. The first step in overcoming amino acid malnutrition in humans, therefore, is to generate DIAAS in food proteins.

Consumption of meat is increasing in the United States of America, in Europe, and in other countries in the developed world (Daniel et al., 2011). Production of pork is increasing globally, and global production reached record levels in 2017 (FAO, 2017). Therefore, because of the widespread production and consumption of pork, it is critical to understand its nutritional quality. As the developing world demands more animal protein, pork's role in furnishing essential amino acids in the diet will increase (Bindari et al., 2017). As a consequence, it is imperative that the protein quality of pork is quantified. Additionally, with the DIAAS determined in this experiment, it is possible to calculate the combined value of cereal grains and pork products as a complete source of amino acids in the diet (Rutherfurd et al., 2015). This concept is important as there is increasing evidence that meat products, when available, often supplant cereal grains to provide a balanced diet (Daniel et al., 2011).

The relatively low DIAAS of all ingredients when calculated for infants is likely of minor importance because pork products are usually not consumed by infants (Bindari et al., 2017). However, the high requirement for Trp in the reference protein for infants combined with the

relatively low concentration of Trp in the ingredients used in this experiment is the reason for the low DIAAS calculated for infants. This observation was also made in hydrolyzed pork samples (Bindari et al., 2017). The sharp drop in the Trp requirement for children from 6 m to 3 y compared with infants, is reflected by large increases in the DIAAS of all proteins and His was the first limiting amino acid in raw pork loin. Nonetheless, based on this requirement pattern, both raw pork loin and casein are considered "good" sources of protein and roasted, grilled, and fried pork loin all qualify as "excellent" sources of protein based on the recommendations made by the FAO (2013). For children that are 3 y and older, raw pork loin also qualifies as a "good" source of protein, whereas all other protein sources qualify as "excellent" sources of protein (FAO, 2013). The relatively lower scores of raw pork loin are of minor importance, however, because pork is rarely consumed raw, and some form of heating is involved in most preparations of pork before consumption.

Values for DIAAS represent concentrations of digestible amino acids weighed against human amino acid requirements, and therefore specifically indicate a protein's capacity to meet amino acid requirements, but do not necessarily directly reflect digestibility of amino acids. However, if protein quality is determined by digestibility of amino acids, pork loin is an exceptionally high quality protein. Indeed, all pork loin products used in this experiment had SID values of greater than 95% for every indispensable and dispensable amino acids. Therefore, the relative bioavailability of amino acids in pork loin products is very high.

Results of this experiment also offer the ability to quantify effects that cooking may have on amino acid digestibility. Cooking methodology varies across cultures and cooking may alter the availability of amino acids in ingredients, particularly via Maillard reactions (Ramadan, 1986; Almeida et al., 2013; Rutherfurd et al., 2015). Although cooking often can have a negative

effect on amino acid concentration and digestibility of foods, data from this experiment demonstrate that cooking may also positively impact protein quality as demonstrated by the fact that DIAAS for cooked pork loins were greater than for raw pork loin, regardless of the reference protein used for calculation. The lack of significant concentrations of reducing sugars in pork likely prevents the Maillard reaction from occurring, and therefore reduces the risk of negative effects on amino acid concentration and digestibility from cooking. However, highly processed pork and bovine muscle hydrosylates that were exposed to very high temperatures have very low DIAAS (Bindari et al., 2017) indicating that heat damage other than Maillard reactions may occur at high temperatures. Regardless of cooking preparation choice, results of this experiment indicate that pork loin can serve in some cases as the sole protein source in a diet fed to young children and adults, and in any case can be used to complement an otherwise amino acid-deficient diet (Rutherfurd et al., 2015).

Values for the AID and SID of amino acids in casein in agreement with reported values (Libao-Mercado et al., 2006; Almeida et al., 2013). The low level of Trp in casein is the reason for the low DIAAS in casein for infants and children up to 36 m. However, for children 3 y and older, the decrease in the Trp requirement resulted in casein having a greater DIAAS than roasted pork loin and raw pork loin, and a DIAAS equivalent to grilled pork loin and fried pork loin. Although casein is a high-quality protein, casein does not represent the complete protein profile of whole milk because casein is what remains after the separation of the whey fraction from milk, and thus represents only a portion of the total amino acid profile of a whole-milk-based product. However, in studies where DIAAS of cooked pork loins are compared with those previously determined in whole milk proteins, they are of similarly high value (Rutherfurd et al., 2015; Mathai et al., 2017).

CONCLUSION

Results of the present research and our previously published data for cereal grains make it possible to calculate DIAAS for a meal rather than for individual food proteins. Additionally, results of this research highlight the differences among cooking procedures on protein quality, and indicates that roasting, grilling, or frying of pork loin increases the quality of protein compared with raw pork loin. Results also indicate that DIAAS values for roasted, grilled, or fried pork loin generally are not different from values obtained from casein.

TABLES

Table 4.1. Analyzed nutrient composition of ingredients[1]

	Ingredient				
Item	Raw pork loin	Roasted pork loin	Grilled pork loin	Fried pork loin	Casein
DM, %	61.43	66.69	68.11	66.70	91.25
CP, %	21.53	28.15	29.08	33.92	90.22
Indispensable amino acids, %					
Arg	2.96	4.00	4.62	4.62	3.99
His	1.09	1.81	1.88	1.99	2.84
Ile	3.30	3.96	4.02	4.12	5.28
Leu	5.01	6.06	6.23	6.51	7.91
Lys	4.10	5.86	6.30	6.82	6.63
Met	1.38	1.95	2.03	2.11	2.38
Phe	2.59	2.98	3.14	3.22	5.15
Thr	2.30	3.42	3.45	3.52	4.25
Trp	0.65	0.85	0.87	1.00	0.84
Val	3.84	4.66	4.58	4.57	6.76
Dispensable amino acids, %					

Table 4.1. (**cont.)**

Ala	4.35	4.87	5.09	4.89	3.80
Asp	4.75	7.62	7.46	7.83	7.06
Cys	0.62	0.93	0.88	0.91	0.55
Glu	10.42	11.37	11.36	11.72	19.18
Gly	3.66	4.29	4.23	4.21	1.99
Pro	2.70	3.90	3.64	3.81	11.91
Ser	1.63	2.65	2.70	2.77	6.05
Tyr	2.87	2.19	2.58	2.77	5.02

[1]Amino acid concentrations expressed on a DM basis.

Table 4.2. Ingredient composition of experimental diets (as-is basis)

Ingredient, %	Diet					
	Raw pork loin	Roasted pork loin	Grilled pork loin	Fried pork loin	Casein	N-free
Raw pork loin	31.71	-	-	-	-	-
Roasted pork loin	-	26.33	-	-	-	-
Grilled pork loin	-	-	26.02	-	-	-
Fried pork loin	-	-	-	21.85	-	-
Casein	-	-	-	-	11.40	-
Soybean oil	3.41	3.68	3.70	3.91	55.40	5.00
Solka floc	2.73	2.95	2.96	3.13	4.00	4.00
Dicalcium phosphate	1.64	1.77	1.78	1.88	1.90	2.40
Limestone	0.34	0.37	0.37	0.39	0.70	0.50
Sucrose	13.66	14.73	14.80	15.63	20.00	20.00
Titanium dioxide	0.27	0.29	0.30	0.31	0.40	0.40
Cornstarch	45.41	48.99	49.20	51.97	55.40	66.50
Magnesium oxide	0.07	0.07	0.07	0.08	0.10	0.10
Potassium carbonate	0.27	0.29	0.30	0.31	0.40	0.40
Sodium chloride	0.27	0.29	0.30	0.31	0.40	0.40
Vitamin mineral premix[1]	0.20	0.22	0.22	0.23	0.30	0.30

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete

Table 4.2. (cont.)

diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 4.3. Analyzed nutrient composition of experimental diets[1]

	Diet				
Item	Raw pork loin	Roasted pork loin	Grilled pork loin	Fried pork loin	Casein
DM, %	79.76	83.93	84.58	85.24	92.39
CP, %	10.00	10.00	10.00	10.00	10.14
Indispensable amino acids, g/kg DM basis					
Arg	10.00	10.79	11.83	9.99	4.15
His	3.70	4.89	4.80	4.30	2.95
Ile	11.14	10.67	10.27	8.91	5.49
Leu	16.92	16.33	15.91	14.09	8.23
Lys	13.81	15.80	16.10	14.77	6.90
Met	4.65	5.25	5.16	4.56	2.47
Phe	8.72	8.03	8.02	6.98	5.36
Thr	7.73	9.20	8.83	7.62	4.42
Trp	2.20	2.29	2.22	2.19	0.88
Val	12.97	12.57	11.69	9.88	7.04
Total	91.84	95.82	94.85	83.29	47.90
Dispensable amino acids, g/kg DM basis					

Table 4.3. (cont.)

Ala	14.65	13.13	13.02	10.58	3.95
Asp	16.01	20.55	19.05	16.93	7.35
Cys	2.09	2.52	2.25	1.98	0.57
Glu	35.13	30.66	29.04	25.37	19.95
Gly	12.35	11.58	10.80	9.12	2.08
Pro	9.12	10.49	9.30	8.25	12.40
Ser	5.49	7.15	6.91	6.00	6.30
Tyr	9.63	5.89	6.61	6.00	5.23
Total	104.48	18.96	96.98	84.23	57.81
Total amino acids	196.31	197.79	191.83	167.52	105.71

[1]Both CP and DM reflect values of diet calculated as they were fed to the animals.

Table 4.4. Apparent ileal digestibility (AID) of amino acids in ingredients

Item	Ingredients					Pooled SEM	*P*-value
	Raw pork loin	Roasted pork loin	Grilled pork loin	Fried pork loin	Casein		
Indispensable amino acids, %							
Arg	95.02[a]	95.68[a]	94.31[a]	91.36[a]	76.20[b]	2.55	<0.05
His	95.07[a]	94.28[a]	94.12[a]	89.42[c]	90.88[b]	0.76	<0.05
Ile	97.24[a]	96.19[a]	95.98[a]	93.47[b]	88.86[c]	0.67	<0.05
Leu	97.20[a]	96.28[a]	96.14[a]	93.79[b]	90.79[c]	0.58	<0.05
Lys	97.24[a]	96.78[a]	97.02[a]	95.22[b]	92.41[c]	0.49	<0.05
Met	98.20[a]	97.69[a]	97.67[a]	96.23[b]	95.15[c]	0.32	<0.05
Phe	96.53[a]	95.23[b]	95.50[ab]	92.86[c]	93.39[d]	0.51	<0.05
Thr	94.01[a]	93.80[a]	93.50[a]	89.77[b]	79.06[c]	0.69	<0.05
Trp	94.68[a]	94.00[ab]	93.46[ab]	91.39[b]	81.97[c]	1.02	<0.05
Val	96.64[a]	95.72[a]	95.27[a]	92.38[b]	89.62[c]	0.77	<0.05
Mean	96.42[a]	95.76[a]	95.59[a]	92.99[b]	88.82[c]	0.70	<0.05
Dispensable amino acids, %							
Ala	96.34[a]	95.62[a]	95.00[a]	91.85[b]	79.45[c]	1.40	<0.05
Asp	95.54[a]	94.87[a]	95.13[a]	91.70[b]	86.37[c]	0.80	<0.05
Cys	89.75[a]	88.26[a]	88.90[a]	85.75[a]	49.49[b]	4.61	<0.05
Glu	97.84[a]	96.68[a]	96.18[a]	93.94[b]	90.55[c]	0.66	<0.05
Gly	90.35[a]	91.55[a]	85.94[a]	81.14[a]	-6.63[b]	9.1	<0.05

Table 4.4. (cont.)

Pro	71.15[a]	75.56[a]	65.90[a]	38.65[b]	39.38[b]	15.55	<0.05
Ser	93.21[a]	93.67[a]	92.85[a]	89.07[b]	84.98[c]	1.30	<0.05
Tyr	97.78[a]	95.39[b]	95.62[b]	92.74[c]	94.79[b]	0.43	<0.05
Mean	93.57[a]	93.45[a]	90.99[ab]	85.67[b]	73.45[c]	2.97	<0.05
Total amino acids	94.91[a]	94.62[a]	93.22[a]	89.35[b]	80.40[c]	1.84	<0.05

[a-g]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]Data are least square means of 6 observations per treatment.

Table 4.5. Standardized ileal digestibility (SID) of amino acids in ingredients[1,2]

Item	Ingredients					Pooled SEM	*P*-value
	Raw pork loin	Roasted pork loin	Grilled pork loin	Fried pork loin	Casein		
Indispensable amino acids, %							
Arg	104.28[a]	104.27[a]	102.14[a]	100.62[ab]	96.79[b]	2.55	<0.05
His	100.49[a]	98.38[b]	98.29[b]	94.08[c]	97.17[b]	0.76	<0.05
Ile	100.48[a]	99.57[a]	99.49[a]	97.51[b]	94.92[c]	0.67	<0.05
Leu	100.47[a]	99.67[a]	99.62[a]	97.71[b]	97.00[b]	0.58	<0.05
Lys	100.12[a]	99.30[a]	99.49[a]	97.92[b]	97.74[b]	0.49	<0.05
Met	99.94[a]	99.23[a]	99.24[a]	98.00[b]	98.18[b]	0.32	<0.05
Phe	100.22[a]	99.24[b]	99.51[ab]	97.47[c]	98.94[b]	0.51	<0.05
Thr	101.92[a]	100.44[b]	100.42[b]	97.79[c]	91.79[d]	0.69	<0.05
Trp	100.19[a]	99.30[ab]	98.91[ab]	96.92[bc]	94.68[c]	1.02	<0.05
Val	100.50[a]	99.65[a]	99.50[a]	97.39[b]	96.11[b]	0.77	<0.05
Mean	100.85[a]	100.01[a]	99.87[a]	97.87[b]	96.67[b]	0.70	<0.05
Dispensable amino acids, %							
Ala	101.23[a]	101.08[a]	100.51[a]	98.63[ab]	96.22[b]	1.40	<0.05
Asp	101.07[a]	99.17[bc]	99.77[ab]	96.92[d]	97.50[cd]	0.80	<0.05
Cys	101.81	98.50	99.39	95.09	89.10	4.75	0.212
Glu	100.72[a]	99.99[a]	99.67[a]	97.94[b]	95.25[c]	0.66	<0.05
Gly	108.03	110.40	106.14	105.06	90.44	9.09	0.330

Table 4.5. (cont.)

Pro	152.15[a]	146.98[ab]	145.34[ab]	128.17[b]	94.45[c]	15.55	<0.05
Ser	103.39[a]	101.49[a]	100.94[ab]	98.42[b]	93.19[c]	1.30	<0.05
Tyr	100.28[a]	99.47[ab]	99.26[b]	96.76[c]	99.05[b]	0.43	<0.05
Mean	106.14[a]	106.33[a]	104.53[a]	101.26[a]	94.43[b]	2.96	<0.05
Total amino acids	103.67[a]	103.31[ab]	102.18[ab]	99.61[b]	95.43[c]	1.84	<0.05

[a-f]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]Data are least square means of 6 observations per treatment.

[2]Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses (g/kg of DMI) amino acids were as follows: Arg, 0.93; His, 0.20; Ile, 0.36; Leu, 0.55; Lys, 0.40; Met, 0.08; Phe, 0.32; Thr, 0.61; Trp, 0.12; Val, 0.49; Ala, 0.72; Asp, 0.88; Cys, 0.24; Glu, 1.01; Gly, 2.18; Ser, 0.56; Tyr, 0.24.

Table 4.6. Comparison of digestible indispensable amino acid score (DIAAS) values[1]

	Ingredients						
Item	Raw pork loin	Roasted pork loin	Grilled pork loin	Fried pork loin	Casein	SEM	*P*-value
DIAAS							
birth to 6 m[2]	55[d] (His)	60[c] (Trp)	61[b] (Trp)	69[a] (Trp)	49[e] (Trp)	0.53	<0.05
6 m to 3 y[3]	78[d] (His)	107[b] (His)	112[a] (His)	112[a] (His)	97[c] (Trp)	1.08	<0.05
3 y and older[4]	97[c] (His)	119[b] (Leu)	123[a] (Leu)	126[a] (Leu)	125[a] (Trp)	1.20	<0.05

[a-f]Means within a row lacking a common superscript letter differ ($P < 0.05$).

[1]First-limiting amino acid is in parantheses.

[2]DIAAS values were calculated using the recommended amino acid scoring pattern for an infant (birth to 6 months). The indispensable amino acid reference patterns are expressed as mg amino acid /g protein: His, 21; Ile, 55; Leu, 96; Lys, 69; Sulfur amino acids, 33; Aromatic amino acids, 94; Thr, 44; Trp, 17; Val, 55 (FAO, 2013).

[3]DIAAS values were calculated using the recommended amino acid scoring pattern for a child (6m to 3y). The indispensable amino acid reference patterns are expressed as mg amino acid /g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; Sulphur amino acids, 27; Aromatic amino acids, 52; Thr, 31; Trp, 8.5; Val, 40 (FAO, 2013).

[4]DIAAS values were calculated using the recommended amino acid scoring pattern for older child, adolescent, and adult. The indispensable amino acid reference patterns are expressed as mg amino acid /g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; Sulphur amino acids, 23; Aromatic amino acids, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

LITERATURE CITED

FAO. 1991. Protein quality evaluation. Report of a joint FAO/WHO expert consultation. FAO food and nutrition paper 51. Food and Agriculture Organization of the United Nations. Bethesda, 1991.

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

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. de Lange. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282-285. doi:10.1016/j.livsci.2007.01.019

Cervantes-Pahm, S. K., Y. Liu, and H. H. Stein. 2014. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br. J. Nutr. 111:1663-1672. doi:10.1017/s0007114513004273

Schaafsma, G. 2012. Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. Br. J. Nutr. 108:S333-S336. doi:10.1017/S0007114512002541

Gilani, G. S. 2012. Background on international activities on protein quality assessment of foods. Br. J. Nutr. 108:S168-S182. doi:10.1017/S0007114512002383

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

WHO. 2007. Protein and amino acid requirements in human nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation. WHO Technical Report Series 935. World Health Organization. Geneva, 2007

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. FAO food and nutrition paper 92. Food and Agriculture Organization of the United Nations. Rome, 2013.

Rowan A.M., P. J. Moughan, M. N. Wilson, K. Maher, and C. Tasman-Jones. 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: 29-42. doi:10.1079/BJN19940108

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

Stein, H. H. , C. Pedersen, A. R. Wirt, R. A. Bohlke. 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J. Anim. Sci. 83:2387-2395. doi:10.2527/2005.83102387x

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC. doi:10.17226/13298

Stein, H. H., C. F. Shipley, and R. A. Easter. 1998. Technical note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1443-1436. doi:10.2527/1998.7651433x

AOAC Int. 2007. Official Methods of Analysis. 18th ed. Rev. 2. W. Howitz, and G. W. Latimer Jr., AOAC Int., Gaithersburg, MD.

Rojas, O. J. and H. H. Stein. 2013. Concentration of digestible and metabolizable energy and digestibility of amino acids in chicken meal, poultry byproduct meal, hydrolyzed porcine intestines, a spent hen-soybean meal mixture, and conventional soybean meal fed to weanling pigs. J. Anim. Sci. 91:3220-3230. doi:10.2527/jas.2013-6269

Schaafsma, G. 2000. The protein digestibility-corrected amino acid score. J. Nutr. 130:1865S-1867S. doi:10.1093/jn/130.7.1865S

Schönfeldt H. C., and N. Hall. 2012. Dietary protein quality and malnutrition in Africa. Br. J. Nutr. 108:S69-S76. doi:10.1017/S0007114512002553

Swaminathan S., M. Vaz, and A. V. Kurpad. 2012. Protein intakes in India. Br. J. Nutr. 108: S50-S68. doi:10.1017/S0007114512002413

Gilani G. S., and E. Sepehr. 2003. Protein digestibility and quality in products containing antinutritional factors are adversely affected by old age in rats. J. Nutr. 133:220-225. doi:10.1093/jn/133.1.220

James L.J., L. Mattin, P. Aldiss, R. Adebishi, and R. M. Hobson. 2014. Effect of whey protein isolate on rehydration after exercise. Amino Acids 46:1217-1224. doi:10.1007/s00726-014-1680-8

McAllan L., P. Skuse, P. D. Cotter, P. O'Connor. J. F. Cryan, R. P. Ross, G. Fitzgerald, H. M. Roche, and K. N. Nilaweera. 2014. Protein quality and the protein to carbohydrate ratio within a high fat diet influences energy balance and the gut microbiota in C57BL/6J mice. PLoS One 2:1-13. doi:10.1371/journal.pone.0088904

Stanstrup J., S. S. Schou, J. Holmer-Jensen, K. Hermansen, and L. O. Dragsted. 2014. Whey protein delays gastric emptying and suppresses plasma fatty acids and their metabolites

compared to casein, gluten and fish protein. J. Proteome Res. 13:2396-2408. doi:10.1021/pr401214w

Stein H. H., N. L. Trottier, C. Bellaver, R. A. Easter. 1999. The effect of feeding level and physiological status on total flow and amino acid composition of endogenous protein at the distal ileum in swine. J. Anim. Sci. 77:1180-1187. doi:10.2527/1999.7751180x

Knabe D.A., D. C. LaRue, E. J. Gregg, G. M. Martinez, and T. D. Tanksley Jr. 1989. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J. Anim. Sci. 67:441-458. doi:10.2527/jas1989.672441x

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

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

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. 127:758-764. doi:10.1093/jn/127.5.758

Gilani G. S., K. A. Cockell, and E. Sepehr. 2005. Effects of antinutritional factors on protein digestibility and amino acid availability in foods. J. AOAC Intl. 88:967-987.

CHAPTER 5: DIGESTIBLE INDISPENSABLE AMINO ACID SCORES (DIAAS) FOR VARIOUS FOOD PROTEINS AND ASSESSMENT OF INTRA-EXPERIMENT VARIATION OF DIAAS

ABSTRACT: An experiment was conducted to test the hypothesis that if 10 different foods that are known to have different protein values are fed to ileal-cannulated pigs, calculated values for digestible indispensable amino acid scores (**DIAAS**) will be different. The second hypothesis was to confirm that DIAAS values calculated from one group of pigs are identical to values calculated from a different group of pigs fed the same diets. The 10 ingredients included: wheat bread, whey protein isolate, zein, sorghum flour, bovine collagen, black beans, pigeon peas, chick peas, roasted peanuts, and Kellogg's® All-Bran®. Thirteen ileal-cannulated gilts were assigned to an incomplete 13 × 6 Latin square design with 13 diets and 6 periods. The 10 ingredients were used to formulate 10 different diets where each ingredient was the sole source of amino acids in the diet. Pigs on treatments 1 to 10 were fed the 10 diets containing the 10 food sources. Pigs on treatments 11, 12, and 13 were fed the whey protein isolate diet, the sorghum diet, and the pigeon pea diet, respectively. These extra replications were used to determine intra-experiment variability. Results indicated that the SID for total amino acids was greater ($P < 0.05$) in toasted wheat bread and sorghum flour than in all other proteins except for chickpeas. The SID for mean indispensable amino acids, mean dispensable amino acids and total amino acids was lower ($P < 0.05$) in All-Bran® than in all other proteins except roasted peanuts. The DIAAS was 0 for zein, bovine collagen, roasted peanuts, and All-Bran® for all reference ratios. Whey protein isolate had the greatest ($P < 0.05$) DIAAS for infants, followed by chickpeas, pigeon peas, sorghum flour, black beans, and toasted wheat bread, in descending order. Whey protein

isolate had the greatest ($P < 0.05$) DIAAS for children (6 m to 3 y) followed by chick peas and pigeon peas. Black beans and sorghum flour had DIAAS values that were not different, but these values were greater ($P < 0.05$) than the DIAAS for toasted wheat bread. Whey protein isolate had the greatest ($P < 0.05$) DIAAS for older children (3 y and older), followed by chickpeas. Pigeon peas had a greater ($P < 0.05$) DIAAS than sorghum flour, which in turn had a greater ($P < 0.05$) DIAAS than black beans, and black beans had a greater ($P < 0.05$) DIAAS than toasted wheat bread. For DIAAS calculated for all 3 reference ratios, there were no differences between replications for whey protein isolate, sorghum, or pigeon peas. The DIAAS values determined in this experiment indicate that most legumes and cereal grain products tested in this experiment are not adequate as the sole sources of protein for humans. Results of this experiment also demonstrate that the pig model is a consistent model for determination of amino acid digestibility and calculation of DIAAS values when different protein sources are used.

Key words: amino acids, protein quality, DIAAS, pigs

INTRODUCTION

The digestible indispensable amino acid score (**DIAAS**) is used to evaluate the protein quality of human foods (FAO, 2013). Values for DIAAS in cereal grains, dairy proteins, soy and pea protein, and protein hydrolysates have been determined (Cervantes-Pahm et al., 2014; Rutherfurd et al., 2015; Mathai et al., 2017; Abelilla et al., 2018; Bindari et al., 2018). However, human diets contain many difference sources of protein and many proteins are used only locally and not throughout the world, but many protein sources have not been characterized in terms of DIAAS values. In addition, most foods are consumed after some kind of preparation, which

often involves thermal treatments, and it is, therefore, necessary to determine DIAAS in foods that are prepared in the form they are consumed by humans.

The pig has been recognized as a preferred model to generate values for DIAAS if data cannot be obtained directly from humans (FAO, 2013), although the rat has been used in some cases (Rutherfurd et al., 2015). Values for DIAAS are relatively easy to calculate based on amino acid digestibility in pigs and because pigs are omnivores they will consume most foods in the same form as humans do. However, to obtain values for DIAAS that can be used in formulation of diets for humans it is necessary that the robustness of the pig model is assessed. There is, however, limited data on the repeatability of data for DIAAS obtained in pigs. Therefore, it was the objective of this experiment to test the hypothesis that values for DIAAS are different if different foods that are known to have different protein values are used. It was the second objective to test the hypothesis that values for DIAAS obtained in 2 different groups of pigs that are fed the same foods are identical.

MATERIALS AND METHODS

Diets, Animals, Housing, and Experimental Design

All animal care procedures were conducted under a research protocol approved by the Institutional Animal Care and Use Committee, University of Illinois, Urbana. A total of 10 different foods were used: wheat bread, whey protein isolate, zein, sorghum flour, bovine collagen, black beans, pigeon peas, chick peas, roasted peanuts, and Kellogg's® All-Bran® (Table 5.1). All ingredients were food grade and procured directly from commercial sources, with the exception of the wheat bread. The 10 ingredients were used to formulate 10 different diets where each ingredient was the sole source of amino acids in the diet (Table 5.2). Each

protein source after their preparation, and immediately before feeding, was combined with a specific mixture of non-protein ingredients. This mixture was specific for each food source and ensured that a balanced diet was provided to the pigs. Titanium dioxide and diatomaceous earth were also included in the non-protein mixture to serve as indigestible markers. In addition, all pigs were fed a N-free diet during one period to determine basal endogenous losses of CP and amino acids for a total of 11 diets. Attempts were made to prepare ingredients in a way that reflected a typical preparation for use in a human diet. Whey protein isolate, zein, and bovine collagen required neither cooking nor processing before feeding and therefore, these ingredients were mixed as-is with non-protein ingredients to provide complete diets.

The bread dough was prepared and baked by the Department of Food Science and Human Nutrition Pilot Processing Plant at the University of Illinois at Urbana-Champaign and followed a standardized recipe (Table 5.3). Sucrose was dissolved in the warmed water and active dry yeast was added to this solution. After yeast activity was confirmed, salt, butter, and flour were added to the solution. Titanium dioxide and diatomaceous earth were also mixed into the solution to ensure even distribution throughout the loaf. Dough was then kneaded in a commercial floor mixer (Hobart Legacy® Mixer, Troy, OH) and portioned into 23 × 13 × 6 cm baking pans where it was allowed to proof for 1 h before being baked at 175°C for 35 minutes. After baking, loaves were cooled to room temperature and then frozen. Immediately before feeding, loaves were thawed, sliced, and toasted lightly in a commercial conveyor toaster (Waring® Commercial CTS1000B, Torrington, CT). After toasting, bread was broken into smaller pieces, mixed with its specific formulation of non-protein ingredients to form a complete diet, and fed to the animals.

Sorghum flour was mixed with water (1:4). The resulting mixture was then boiled on a stovetop for approximately 20 min until a thick, paste-like consistency was achieved; preparation took place in large batches to ensure consistency among feedings. The cooked flour was mixed with the specific formulation of non-protein ingredients for this diet, and fed to the animals.

Black beans were procured in the dry form and were soaked in water at room temperature for 18 h prior to cooking. After soaking, black beans were strained and weighed. Table salt was added, at 720 mg per 100 g black beans, in solution with water, and beans were then pressure-cooked in a pressure cooker (ALL-AMERICAN®, Model No. 921, Wisconsin Aluminum Foundry Company, WS) at a pressure of approximately 100 kPa and a temperature of 121°C for a period of 20 min. Cooked black beans were blended with the non-protein ingredients to form the complete diet. Approximately 200 mL of water was then added to the diet to improve consistency and this mixture was mashed with a potato masher hand tool (Farberware® Potato Masher, Model# 5124791, Garden City, New York) before being fed to the animals.

Pigeon peas were procured in the dry form and were prepared for feeding as outlined for black beans with the exception that they were pressure-cooked for only 10 min. The cooked pigeon peas were blended with the non-protein ingredients to form a complete diet immediately before feeding. Chickpeas were procured in canned form. After straining off of water, chickpeas and 200 mL of water were blended using a food processor (Waring® Commercial WFP16S, Torrington, CT) until a consistent texture was achieved. Chickpeas were blended with the specific formulation of non-protein ingredients to form a complete diet immediately before feeding.

Peanuts were obtained in the de-shelled and roasted form. Peanuts were coarsely ground by Department of Food Science and Human Nutrition Pilot Processing Plant at the University of

Illinois at Urbana-Champaign. Ground peanuts were blended with the non-protein ingredients that were needed to form a complete diet. After mixing, the peanut diet mixture was mixed with approximately 200 mL of water to improve consistency before feeding.

Kellogg's® All-Bran® was combined with the non-protein ingredients needed to formulate a balanced diet. After mixing, approximately 400 mL of water was added to the mixture to moisten the cereal and to ensure even distribution of the non-protein mixture.

Thirteen gilts (initial BW: 29.09 ± 3.26 kg) of PIC Line 03 genetics were surgically fitted with T-cannulas in the distal ileum (Stein et al., 1998). After surgery, pigs were housed individually in pens (2 × 3m) that had a concrete half-slatted floor and were equipped with a feeder and a nipple drinker. Pigs were fed a nutritionally adequate diet based on toasted wheat bread (Table 5.4) for one week following surgery to allow time for surgical recovery and to adapt the animals to a human diet. Pigs were then allotted to an incomplete 13 × 6 Latin square design with 13 diets and six, 7 d periods comprising the rows and the columns of the square. Pigs on treatments 1 to 10 were fed the 10 diets containing the 10 food sources. Pigs on treatments 11, 12, and 13 were fed the whey protein isolate diet, the sorghum diet, and the pigeon pea diet, respectively. These additional replications were identified as separate treatments that were used to test the hypothesis that values for digestibility of amino acids and DIAAS are repeatable within an experiment. Therefore, during each of the Latin square periods, there were 13 pigs on treatment with one pig fed each diet with the exception that 2 replicates were fed the whey protein isolate, sorghum flour, and pigeon pea diets. After the initial 3 periods, all 13 pigs were fed a N-free diet for one period. This period was placed in the middle of the study to balance residual experimental effects on basal endogenous losses of amino acids. After this period, pigs were eased back into their normal treatments by feeding for 1 week the toasted wheat bread

based diet that was also fed immediately after surgery. Pigs were then returned to the second half of the Latin square.

Data Recording and Sample Collection

Pigs were fed their respective diets in quantities equivalent to 8% of their metabolic body weight ($BW^{0.60}$). The daily feed allotment was provided in the form of 2 equal daily meals at 0800 and 1600 h. Water was available at all times. Each period lasted 7 d. The initial 5 d of each period was considered an adaptation period to the diet and ileal digesta samples were collected following standard procedures (Stein et al., 1998). Briefly, cannulas were opened and cleaned, a plastic bag was attached to the cannula barrel, and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta or at least once every 30 min, and immediately frozen at – 20°C to prevent bacterial degradation of the amino acids in the digesta. Samples were collected for 9 h each day starting immediately after feeding the morning meal. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment and these weights were used to calculate the provision of feed during the subsequent period. No digesta samples were collected during the adaptation period immediately following the N-free treatment period.

Chemical Analysis

Ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized and ground using a coffee grinder (Hamilton Beach® Model 80335, Glen Allen, VA). Diets, ingredients, and all ileal digesta samples were analyzed for dry matter (**DM**; Method 927.05; AOAC International, 2007) and CP (Method 990.03; AOAC International, 2007) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI) at the Monogastric Nutrition Laboratory

at the University of Illinois. Samples were analyzed in duplicate, but analyses were repeated if the analyzed values were more than 5% apart. All samples were also analyzed for amino acids [Method 982.30 E (a, b, c); AOAC International, 2007], and samples of diets and ileal digesta were analyzed for Ti (Myers et al., 2004).

Calculations

Apparent ileal digestibility values for amino acids in the protein sources were calculated using equation [6] (Stein et al., 2007):

$$AID\ (\%) = [1 - [(AAd/AAf) \times (Tif/Tid)] \times 100 \quad \textbf{[6]}$$

where AID is the apparent ileal digestibility of an amino acids (%), AAd is the concentration of that amino acid in the ileal digesta DM, AAf is the amino acid concentration of that amino acid in the feed DM, Tif is the titanium concentration in the feed DM, and Tid is the titanium concentration in the ileal digesta DM.

The basal endogenous flow to the distal ileum of each amino acid was determined based on the flow obtained after feeding the N-free diet using equation [7] (Stein et al., 2007):

$$IAA_{end} = [AAd \times (Tif/Tid)] \quad \textbf{[7]}$$

where IAA_{end} is the basal endogenous loss of an amino acid (mg per kg DM intake). The basal endogenous loss of CP was determined using the same equation.

By correcting the AID for the IAA_{end} of each amino acid, standardized ileal amino acid digestibility values were calculated using equation [8] (Stein et al., 2007):

$$SID = [(AID + IAA_{end})/AAf] \quad \textbf{[8]}$$

where SID is the standardized ileal digestibility value (%).

The concentration of SID amino acids (g/kg) in each ingredient was calculated by multiplying the SID value (%) for each amino acid by the concentration (g/kg) of that amino acid

in the ingredient, and this value was then divided by the concentration of CP in the ingredient to calculate digestible indispensable amino acid (mg) in 1 g protein (Cervantes-Pahm et al., 2014; Mathai et al., 2017). The digestible indispensable amino acid reference ratios were calculated for each ingredient using the following equation [9] (FAO, 2013):

Digestible indispensable amino acid reference ratio = digestible indispensable amino acid content in 1 g protein of food (mg) / mg of the same dietary indispensable amino acid in 1g of reference protein. **[9]**

The reference proteins were based on FAO (2013) definitions and separate ratios were calculated using the reference protein for infants less than 6 months old, children from 6 months old to 36 months old, and children older than 36 months old, adolescents, and adults. The DIAAS were then calculated using the following equations [10] (FAO, 2013):

DIAAS (%) = 100 × lowest value of digestible indispensable amino acid reference ratio. **[10]**

Statistical Analyses

Normality of data was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Inst. Inc., Cary, NC). Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the pig as the experimental unit. The statistical model to determine differences in SID of amino acid values among ingredients included diet as the main effect and pig and period as random effects. Treatment means were calculated using the LSMEANS statement, and when significantly different, means were separated using the PDIFF option of the MIXED procedure. An ANOVA was conducted to determine intra-replication differences in DIAAS between the 2 replicates of pigs fed the diets

containing whey protein isolate, sorghum flour, and pigeon peas. Significance for all analyses was considered as $P < 0.05$.

RESULTS

All pigs remained healthy throughout the experiment. Diets were consumed readily and completely by all animals. The mean AID for indispensable amino acids was greater ($P < 0.05$) in toasted wheat bread and whey protein isolate than in bovine collagen, zein, All-Bran®, and roasted peanuts in that order (Table 5.6). The mean AID for dispensable amino acids was greatest ($P < 0.05$) in toasted wheat bread. The AID for mean indispensable amino acids and total amino acids was lower ($P < 0.05$) in All-Bran® than in all other proteins except roasted peanuts.

The SID for total amino acids was greater ($P < 0.05$) in toasted wheat bread and sorghum flour than in all other proteins except for chickpeas (Table 5.7). The SID for mean indispensable amino acids, mean dispensable amino acids and total amino acids was lower ($P < 0.05$) in All-Bran® than in all other proteins except roasted peanuts.

The DIAAS was 0 for zein, bovine collagen, roasted peanuts, and All-Bran® for all reference ratios. Whey protein isolate had the greatest ($P < 0.05$) DIAAS for infants, followed by chickpeas, pigeon peas, sorghum flour, black beans, and toasted wheat bread, in descending order (Table 5.8).

Whey protein isolate had the greatest ($P < 0.05$) DIAAS for children (6 m to 3 y) followed by chick peas and pigeon peas. Black beans and sorghum flour had DIAAS values that were not different, but these values were greater ($P < 0.05$) than the DIAAS for toasted wheat bread.

Whey protein isolate had the greatest ($P < 0.05$) DIAAS for older children (3 y and older), followed by chickpeas. Pigeon peas had a greater ($P < 0.05$) DIAAS than sorghum flour, which in turn had a greater ($P < 0.05$) DIAAS than black beans, and black beans had a greater ($P < 0.05$) DIAAS than toasted wheat bread.

The SID of Met in whey protein isolate was lower ($P < 0.05$) for replicate 1 than in replicate 2. However, for all other amino acids there were no differences between replications regardless of feed ingredient (Table 5.9). For DIAAS calculated for all 3 reference ratios, there were no differences between replications for whey protein isolate, sorghum, or pigeon peas (Table 5.10).

For DIAAS for all reference ratios used in this experiment, Lys was the first limiting amino acid in toasted wheat bread, zein, sorghum flour, and All-Bran®. The sulfur amino acids, Trp, and Thr were the first limiting amino acids for the DIAAS for all reference ratios for black beans, bovine collagen, and roasted peanuts, respectively. For both pigeon peas and chick peas, the first-limiting amino acid for the DIAAS for children from birth to 6 m was Trp, however, this changed to the sulfur amino acids for DIAAS for children 6 m to 3 y and for those 3 y and older. For the DIAAS for whey protein isolate from birth to 6 m, the first-limiting amino acid was the aromatic amino acids, however, for DIAAS for children 6 m to 3 y and for those 3 y and older, the first-limiting amino acid was His.

DISCUSSION

One of the primary objectives of this experiment was to establish DIAAS for several significantly different proteins and to determine how the DIAAS methodology describes proteins of vastly different origins. Each ingredient used in this experiment was distinct, but was chosen to reflect protein sources used in diets around the globe. FAO recommendations for nutrient

claims describe DIAAS below 75 to represent ingredients that cannot make any claims regarding protein quality, DIAAS between 75 and 100 may be described as "good' sources of protein, and DIAAS greater than 100 can be described as "excellent/high" quality sources of protein. Based on the results of this experiment, only whey protein isolate when fed to children above 3 y can make the claim to be an excellent protein source. When fed to children between 6 m and 3 y, whey protein isolate can be considered a "good" source of protein. Chickpeas can be considered a "good" protein source when fed to children above 6 m of age. All other protein sources used in this experiment, based on FAO guidelines, can make no claims to their protein quality. This observation is significant because several protein sources used in this experiment are major staple foods in some countries.

Commonly referred to as legumes, 4 of the 10 ingredients used in this experiment are representatives of the *Fabacaea* family: black beans, pigeon peas, chickpeas, and roasted peanuts. Combined, in 2013, 121 million metric tons of legumes (excluding soybean) were produced (Foyer et al, 2016). As N fixers, legumes play an important role in rural agriculture. In addition to their use as food crops, legumes have been proposed as a potential solution to the problem of eutrophication of soils due to N fertilization and rural farmers in developing countries often alternate fields between legume and grain production (FAO, 1999a).

The *Phaseolus* genus represents the majority of common beans. In particular, *Phaseolus vulgaris*, of which the black bean is a variety, represents "the most important food legume for direct consumption in the world" (FAO, 1999a; Joshi and Rao, 2017; Izquierdo et al., 2018). In part, this is due to the widespread distribution, relative ease to grow, and the long storage life of *Phaselous vulgaris* (FAO, 1999a). In addition, beans have long been considered a high nutrition food and are often cited for their high mineral content (Izquierdo et al., 2018) and protein

content, particularly when combined with other staples such as rice, wheat, or corn (FAO, 1999a; Joshi and Rao, 2017).

In terms of protein, the black bean contains approximately 22% CP, and when compared with other plant proteins it has a relatively high concentration of indispensable amino acids. The first limiting amino acid in black beans was the sulfur amino acids, which indicates that black beans may complement corn to balance diets because corn has a relatively high concentration of sulfur amino acids. However, as results of this experiment demonstrate, the availability of amino acids in black beans appears to be low.

Pigeon peas (*Cajanus cajan*) are cultivated on approximately 4.79 Mha, with considerable production in Africa, India, and Southeast Asia (Saxena et al., 2010b). Pigeon peas are the second-most commonly grown pulse in India (second to chickpea) and are a common component of the daily diet in India (Saxena et al., 2010a). Pigeon peas are used in most households to complement cereals in the diet and meet protein needs (Ofuya and Akhidue, 2005; Saxena et al., 2010a). However, it is estimated that in some developing countries, protein is often available at one-third of the requirement (Saxena et al., 2010a; 2010b), and it has been estimated that nearly 20% of the protein available to man originates from pulses (Reddy et al., 1985; Ofuya and Akhidue, 2005). In consideration of the critical role that pulses play in meeting protein requirements, the low DIAAS of pigeon peas indicates that pigeon peas by themselves will not provide a balanced diet due to the low concentration of sulfur amino acids. It is, therefore, important that pigeon peas and other pulses are combined with foods that have greater concentrations of sulfur amino acids to provide a balanced meal. There is limited information on the DIAAS of pigeon peas, however, the DIAAS of pea protein concentrate (*Pisum sativum*) has been determined (Mathai et al., 2017). Although from a difference genus, the field pea is also a

legume. The pigeon peas in our experiment are limited by the same amino acids (Trp and SAA), and follow a similar trend across the reference patterns, as was observed for pea protein concentrate. Nevertheless, pea protein concentrate is a purified product with nearly twice as much CP as pigeon peas and the DIAAS, therefore, is greater in pea protein concentrate than in pigeon peas.

The greater DIAAS of chickpeas (*Ciser arietinum*) than for the other legumes used in this experiment may be because chickpeas was the only legume included in this experiment that was canned, and therefore precooked and stored in brine until preparation immediately before feeding. However, the amino acid composition of chickpeas is similar to that in black beans and pigeon peas and the digestibility values for chickpeas were not different from those of pigeon peas, but greater than those for black beans. As a result, the higher DIAAS values for chickpeas than for black beans appears to reflect not only the higher digestibility values, but also a more favorable amino acid composition of chickpeas in relation to the reference pattern. Pigeon peas and chickpeas both have the same first-limiting amino acid for each reference pattern. The higher scores for chickpeas are the result of not only the higher concentration of both Trp and SAA, but also due to the high SID values for both of those amino acids. Although, it can be assumed that the canning method did not alter the amino acid composition of the chickpeas, it is possible that the canning process may have increased amino acid digestibility.

Although a member of the *Fabaceae* family, the peanut (*Arachis hypogaea)* is considered a legume, but not a pulse according to the FAO (FAOSTAT, 1994). Pulses are defined as legumes harvested only for the dry-grain, whereas the peanut is harvested for both its grain and its oil. This definition may be an important consideration in terms of protein availability. Despite a higher concentration of CP than in all other legumes used in this study, the concentration of

amino acids was substantially lower, which in combination with the low SID of amino acids is the reason for the low DIAAS values that were calculated for peanuts. The reason for the low SID of amino acids in roasted peanuts most likely is that the grinding that was used resulted in chunks of peanuts being fed and this likely prevented the digestive enzymes from hydrolyzing all the peptide bonds. However, the coarse grinding was chosen to feed peanuts in the form that they are likely to be consumed by humans.

The FAO forecast for 2018 global production of cereal grains (Family *Gramineae*) is approximately 2.6 billion metric tons (FAO, 2018). Despite being close to the record production of 2017, this number is below the expected utilization of cereal grains in 2018, and utilization of cereal grain is predicted to continue to increase in the future (FAO, 2018). Sorghum is considered a subsistence crop and is grown throughout the world due to its ability to grow in semi-arid conditions (FAO, 1999b). As a result, sorghum is a major staple crop grown in some countries in Africa and is commonly consumed in porridges, breads, and in various malted forms (FAO, 1999b). Wheat is grown and consumed throughout the world and has been a staple food in Europe, Asia, and Africa for over 8 millennia (FAO, 1999). Currently, wheat is grown on more land than any other commercialized crop and it is considered the most important food grain for humans (FAOSTAT, 2014). Kellogg's® All-Bran® is a wheat-based cereal, but unlike the wheat bread it is made from the bran of wheat.

Both the sorghum flour and the wheat bread represent foods made from the endosperm portion of grains (FAO, 1999b). The endosperms of cereal grains are primarily composed of starch, are low in fiber, and are low in protein, whereas bran products are typically much higher in fiber, contain little starch, and have slightly more protein than endosperm products (FAO, 1999b; Liu and Ng, 2014; Casas and Stein, 2016). Our analyzed values for CP in these

ingredients reflects this with All-Bran® having a greater CP concentration than the wheat bread and sorghum flour. However, the fractionation of the cereal grain has important implications for the digestibility of the resulting products. Fiber can negatively influence amino acid availability (Mathai et al., 2015). Highly processed finely-ground flour products such as sorghum flour and the wheat flour used to make the wheat bread represent highly digestible, low fiber products which is the reason for the high SID in these foods. The SID values for wheat bread and sorghum flour in this experiment are also higher than those reported for whole wheat and whole sorghum (Cervantes-Pahm et al., 2014; Mathai et al., 2017), which is likely due to the lower fiber concentration in the flour. Although the SID values were higher, the DIAAS values for toasted wheat bread were similar to those determined for whole wheat by Cervantes-Pahm et al. (2014) and lower than those determined by Mathai et al. (2017), which is due to the lower concentration of amino acids in the flour compared with the whole grain. Cooking of the flours may also have altered the protein structures, thereby increasing digestibility.

The processing of wheat bran to produce All-Bran® may have affected the digestibility of some amino acids. Heat damage during processing and Maillard reactions are likely the reason for the extremely low SID of Lys in All-Bran®, but the SID of Lys in corn bran is also close to zero (Liu et al., 2014) as was observed for wheat bran in this experiment. However, of likely greater impact on the digestibility of amino acids, is the high fiber content of All-Bran®. Indeed, one 50 g serving of All-Bran® contains 16 g of Dietary Fiber. This high fiber content will cause All-Bran® to induce high specific endogenous losses of amino acids, and therefore, will reduce the SID of all amino acids (Urriola et al., 2013). The high fiber content may also inhibit enzymatic hydrolysis of the wheat bran proteins, which is likely to contribute to the low protein

quality of All-Bran®. For these reasons, All-Bran® appears to not be an effective protein source in the food.

Zein is one of the main proteins in corn, and is typically used in industrial settings as a polymeric compound as opposed to as a food source (Shukla and Cheryan, 2001). Despite collagen being the most abundant protein in the human body (Di Lullo et al., 2002), bovine collagen is rarely consumed directly as a source of protein, but it may be consumed on a supplemental basis (Song et al., 2017). The low concentration of Lys and Trp in zein and the low Trp concentration in bovine collagen precludes them from having high DIAAS values because DIAAS values are determined by the first limiting amino acid. Therefore, DIAAS values reflect a protein's ability to serve as the sole source of protein in a diet. Accordingly, zein and bovine collagen need to be combined with other proteins that have high concentrations of Lys and Trp to provide a balanced diet.

The high DIAAS of whey protein isolate is in agreement with values reported for whey proteins (Mathai et al., 2017). Although the first-limiting amino acid is the same, the DIAAS values determined in this experiment are significantly lower than reported values because of a lower digestibility of amino acids in the whey protein isolate used in this experiment. There was also lower concentration of His in the isolate used in this experiment, and because His is the first-limiting amino acid for the DIAAS for two of the reference patterns, this resulted in the lower DIAAS values in this experiment.

One of the objectives of this experiment was to compare the intra-experiment variation in the measurement of digestibility of amino acids and in the subsequent calculation of DIAAS. For the 3 replicated proteins there were no differences in the digestibility of amino acids with the exception of Met in whey protein isolate, for which the difference was less than 2%. There were

no differences in DIAAS calculated for the proteins between replicated groups. Therefore, based on the results of these analyses, it appears that the determination of SID values for amino acids and DIAAS of human foods using pigs is repeatable model.

CONCLUSION

The results of this experiment indicate that the DIAAS of legumes tested in this experiment can be variable. In particular, results indicate that of the legumes tested, chickpeas are the superior protein source in a human diet. Results also indicate that although cereal grain products can have high a digestibility of amino acids, their low concentration of amino acids limits their value as protein sources in the diet. Results of this experiment also demonstrate that the pig model is a consistent model for determination of amino acid digestibility and DIAAS determination even when disparate protein sources are used.

TABLES

Table 5.1. Analyzed nutrient composition of foods[1]

Item	Toasted wheat bread	Whey protein isolate	Zein	Sorghum flour	Bovine collagen	Black beans	Pigeon peas	Chick peas	Roasted peanuts	All-Bran®
DM, %	90.32	96.43	96.56	88.20	97.04	96.23	95.52	31.18	95.50	97.82
CP, %	11.94	86.44	94.17	9.77	106.55	21.73	25.8	22.19	30.49	14.91
Indispensable amino acids, %										
Arg	0.42	1.94	1.66	0.39	8.56	1.34	1.95	1.82	3.78	0.75
His	0.27	1.56	1.19	0.24	0.78	0.61	0.62	0.54	0.71	0.35
Ile	0.50	6.92	4.37	0.45	1.68	1.04	1.16	1.08	1.15	0.50
Leu	0.87	9.77	19.77	1.41	3.16	1.69	1.86	1.79	2.02	0.89
Lys	0.30	8.92	0.05	0.25	4.12	1.51	1.76	1.46	1.05	0.40
Met	0.19	2.16	1.73	0.19	0.88	0.25	0.20	0.31	0.31	0.18
Phe	0.62	2.82	6.84	0.57	2.27	1.23	1.30	1.40	1.61	0.59
Thr	0.34	6.90	2.75	0.33	1.81	0.91	0.90	0.80	0.79	0.43
Trp	0.16	1.78	0.04	0.11	0.01	0.18	0.20	0.23	0.34	0.18

Table 5.1. (cont.)

Val	0.54	6.05	3.95	0.56	2.46	1.17	1.28	1.08	1.30	0.67
Dispensable amino acids, %										
Ala	0.39	5.02	9.69	0.95	9.55	0.88	1.06	0.94	1.18	0.62
Asp	0.52	9.93	5.44	0.69	6.09	2.50	2.89	2.48	3.58	0.92
Cys	0.28	2.13	0.89	0.22	0.06	0.26	0.28	0.29	0.41	0.27
Glu	4.22	17.46	24.44	2.15	10.47	3.17	4.08	3.33	5.60	3.01
Gly	0.47	1.47	1.07	0.34	23.62	0.84	1.01	0.86	1.77	0.72
Pro	1.41	6.41	9.34	0.86	14.11	0.90	0.96	0.91	1.25	0.97
Ser	0.54	3.88	4.78	0.41	2.85	1.03	1.05	0.99	1.28	0.50
Tyr	0.25	2.63	4.99	0.29	0.83	0.67	0.75	0.64	1.25	0.35
Total amino acids	12.28	97.74	102.99	10.41	93.33	20.19	23.30	20.95	29.37	12.30

[1]Values for CP and amino acids are expressed on a DM basis.

Table 5.2. Ingredient composition of experimental diets (as-is basis)

Ingredient, %	Diet										
	Toasted wheat bread	Whey protein isolate	Zein	Sorghum flour	Bovine collagen	Black beans	Pigeon peas	Chick peas	Roasted peanuts	All-Bran®	N-free
Protein source	96.05	11.30	11.70	94.50	10.20	44.80	48.80	52.20	35.7	80.30	-
Corn starch	-	65.10	62.80	-	66.20	31.70	27.70	24.70	40.80	-	76.40
Sucrose	-	10.00	10.00	-	10.00	10.00	10.00	10.00	10.00	-	10.00
Cellulose	-	3.00	3.00	-	3.00	3.00	3.00	3.00	3.00	7.25	3.00
Canola oil	-	5.00	5.00	-	5.00	5.00	5.00	5.00	5.00	7.25	5.00
L-Lysine HCl	-	-	1.20	-	-	-	-	-	-	-	-
L-Tryptophan	-	-	0.20	-	-	-	-	-	-	-	-
Dicalcium phosphate	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50

Table 5.2. (cont.)

Limestone	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.30	0.30	0.50	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Sodium chloride[2]	-	0.40	0.40	0.40	0.40	0.40	0.40	-	0.40	0.10	0.40
Magnesium oxide	-	0.10	0.10	-	0.10	-	-	-	-	-	0.10
Potassium carbonate	0.70	0.70	1.00	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Titanium dioxide	-	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Diatomaceous earth	-	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Vitamin mineral mixture[1]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete

Table 5.2. (cont.)

diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[2] For the black bean and pigeon pea treatments, this value also accounts for the salt added during the pressure cooking process.

Table 5.3. Ingredient composition of toasted wheat bread (as-is basis)

Ingredient	%
Bleached whole wheat flour	51.50
Sucrose	9.49
Active dry yeast	0.91
Salt	0.61
Butter	4.04
Water	32.31
Titanium dioxide	0.40
Diatomaceous earth	0.74
Total	100

Table 5.4. Ingredient composition of basal adaptation diet (as-is basis)

Ingredient	%
Toasted wheat bread	68.80
Casein	7.00
Wheat gluten meal	5.00
Whey powder	5.00
Potato protein	3.00
Skim milk powder	2.00
Canola oil	5.00
Dicalcium phosphate	1.70
Calcium carbonate	0.50
Potassium carbonate	0.30

Table 5.4. (cont.)

Sodium bicarbonate	0.50
L-Lysine HCl	0.20
Vitamin mineral mixture[1]	1.00
Total	100.00

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 5.5. Analyzed nutrient composition of experimental diets

Item	Toasted wheat bread	Whey protein isolate	Zein[1]	Sorghum flour	Bovine collagen	Black beans	Pigeon peas	Chick peas	Roasted peanuts	All-Bran®
DM, %	90.97	90.98	91.17	88.72	90.97	43.06	44.72	45.48	92.86	97.42
Indispensable amino acids, g/kg DM										
Arg	3.66	2.31	1.97	3.20	8.90	5.87	9.50	9.55	12.95	6.04
His	2.31	1.98	1.54	1.97	0.88	2.68	3.01	2.83	2.44	2.81
Ile	4.33	8.79	5.59	3.76	1.98	4.55	5.67	5.67	3.95	4.06
Leu	7.61	12.42	25.01	11.66	3.85	7.42	9.09	9.39	6.92	7.20
Lys	2.60	11.21	0.06	2.07	4.40	6.60	8.58	7.66	3.59	3.23
Met	1.64	2.64	2.08	1.60	0.99	1.09	0.97	1.63	1.08	1.49
Phe	5.39	3.63	8.67	4.70	2.64	5.37	6.34	7.35	5.52	4.80

Table 5.5. (cont.)

Thr	2.98	8.68	3.51	2.73	2.09	4.00	4.39	4.20	2.69	3.48
Trp	1.35	1.87	0.01	0.94	0.11	0.77	0.97	1.21	1.15	1.49
Val	4.72	7.58	5.05	4.61	2.86	5.14	6.23	5.67	4.45	5.46
Total	36.58	61.12	64.94	37.23	28.69	43.50	54.77	55.14	44.72	40.07
Dispensable amino acids, g/kg DM										
Ala	3.37	6.49	12.29	7.90	10.77	3.87	5.16	4.93	4.05	5.05
Asp	4.52	12.75	7.02	5.74	7.04	10.97	14.10	13.01	12.27	7.45
Cys	2.41	2.75	1.10	1.79	0.11	1.14	1.38	1.52	1.40	2.15
Glu	36.68	22.53	30.71	17.86	12.20	13.88	19.93	17.47	19.19	24.34
Gly	4.04	1.98	1.43	2.82	27.26	3.69	4.90	4.51	6.06	5.80
Pro	12.23	7.91	11.74	7.15	15.83	3.96	4.70	4.77	4.27	7.86

Table 5.5. (cont.)

Ser	4.72	5.17	5.70	3.38	3.30	4.50	5.11	5.19	4.38	4.06
Tyr	2.21	2.53	4.94	2.44	0.99	2.91	3.68	3.36	4.27	2.81
Total	70.18	62.10	74.92	49.08	77.50	44.91	58.96	54.77	55.88	59.52
Total amino acids	106.77	123.22	139.85	86.31	106.19	88.41	113.73	109.91	100.60	99.59

[1]Lys and Trp values are based on calculation due to the addition of synthetic sources to the diet.

Table 5.6. Apparent ileal digestibility (AID) of amino acids in foods

Item	Toasted wheat bread	Whey protein isolate	Zein	Sorghum flour	Bovine collagen	Black beans	Pigeon peas	Chick peas	Roasted peanuts	All-Bran®	Pooled SEM	*P*-value
Indispensable amino acids, %												
Arg	78.7^{ab}	54.0^{c}	26.9^{d}	80.0^{ab}	85.4^{ab}	72.7^{b}	87.7^{a}	89.0^{a}	55.7^{c}	51.9^{c}	5.6	<0.05
His	84.3^{a}	79.8^{a}	50.5^{c}	81.7^{a}	54.8^{bc}	59.6^{b}	76.7^{a}	77.9^{a}	17.9^{e}	38.4^{d}	3.3	<0.05
Ile	84.2^{ab}	88.0^{a}	58.4^{c}	80.5^{ab}	66.3^{c}	59.1^{c}	76.7^{b}	75.7^{b}	-0.5^{e}	29.9^{d}	3.3	<0.05
Leu	85.8^{a}	89.2^{a}	65.5^{d}	85.2^{ab}	67.2^{cd}	58.4^{d}	75.4^{c}	76.2^{bc}	9.5^{f}	35.6^{e}	3.7	<0.05
Lys	58.6^{c}	86.5^{a}	-1,546.7	59.1^{c}	68.1^{bc}	67.6^{c}	82.1^{a}	79.9^{ab}	-48.6^{e}	-19.8^{d}	4.5	<0.05
Met	87.7^{a}	89.7^{a}	63.1^{cd}	87.7^{a}	71.1^{bc}	55.1^{d}	67.7^{c}	79.0^{ab}	0.90^{f}	38.9^{e}	4.1	<0.05
Phe	87.7^{a}	80.4^{ab}	61.9^{d}	82.9^{ab}	70.7^{cd}	62.8^{d}	78.5^{bc}	81.7^{ab}	22.7^{f}	41.7^{e}	3.4	<0.05
Thr	69.4^{a}	71.2^{a}	49.0^{bc}	63.7^{a}	46.8^{c}	37.8^{c}	63.0^{a}	60.4^{ab}	-65.5^{e}	-8.6^{d}	4.5	<0.05
Trp	85.5^{a}	86.8^{a}	-374.1	79.7^{ab}	-62.9	44.0^{d}	61.1^{c}	71.9^{bc}	14.7^{e}	32.6^{d}	4.7	<0.05

Table 5.6. (cont.)

Val	77.6^{a}	76.2^{a}	53.3^{cd}	74.1^{a}	61.8^{bc}	44.5^{d}	70.0^{ab}	67.0^{ab}	-19.7^{f}	18.0^{e}	4.2	<0.05
Mean	80.8^{a}	82.0^{a}	57.8^{c}	79.0^{ab}	70.5^{b}	57.3^{c}	76.0^{ab}	77.4^{ab}	12.2^{e}	27.8^{d}	3.5	<0.05
Dispensable amino acids, %												
Ala	71.2^{ab}	71.7^{ab}	60.8^{b}	82.9^{a}	82.8^{a}	37.6^{c}	64.6^{b}	66.5^{b}	-26.8^{e}	14.8^{d}	4.6	<0.05
Asp	67.9^{c}	84.4^{a}	53.9^{d}	75.4^{abc}	46.5^{d}	51.6^{d}	77.7^{ab}	69.3^{bc}	18.0^{e}	-9.8^{f}	3.7	<0.05
Cys	83.1^{a}	84.4^{a}	41.8^{b}	74.8^{a}	-254.6	-12.7^{c}	46.8^{b}	46.5^{b}	-22.9^{c}	-10.0^{c}	5.1	<0.05
Glu	94.3^{a}	83.0^{bc}	64.6^{d}	84.5^{b}	75.9^{c}	65.2^{d}	82.5^{bc}	80.1^{bc}	36.6^{e}	65.3^{d}	2.9	<0.05
Gly	49.7^{ab}	-30.5^{c}	-72.7^{d}	49.4^{ab}	69.6^{a}	-3.3^{c}	30.5^{b}	31.6^{b}	-31.1^{c}	-23.6^{c}	15.6	<0.05
Pro	62.7^{ab}	-25.7^{c}	6.8^{abc}	79.0^{a}	41.1^{abc}	-4.2^{bc}	3.5^{bc}	54.2^{ab}	-224.9^{d}	-21.7^{c}	31.6	<0.05
Ser	82.9^{a}	65.6^{bcd}	59.9^{cd}	72.5^{b}	58.0^{de}	49.6^{e}	69.0^{bc}	72.7^{b}	-2.5^{g}	16.9^{f}	4.3	<0.05
Tyr	78.4^{a}	76.1^{a}	60.3^{b}	75.1^{a}	42.8^{c}	56.0^{b}	73.4^{a}	75.1^{a}	29.1^{d}	28.5^{d}	3.7	<0.05

Table 5.6. (cont.)

Mean	82.3[a]	62.8[cd]	50.8[de]	77.2[ab]	65.4[bc]	43.6[e]	66.1[bc]	67.2[bc]	-5.0[g]	24.5[f]	5.3	<0.05
Total amino acids	82.0[a]	72.3[ab]	53.7[c]	77.7[ab]	67.1[b]	50.3[c]	70.8[b]	72.2[ab]	2.6[e]	26.1[d]	4.3	<0.05

[a-g]Means within a row lacking a common superscript letter differ ($P < 0.05$). Values lacking superscript were not included in Fisher's Least Significant Difference analysis or mean amino acids and total amino acids least squares means estimations.

[1]Data are least square means of 6 observations per treatment; with the exception of the whey protein isolate, sorghum, and pigeon peas treatments for which there are 12 observations.

Table 5.7. Standardized ileal digestibility (SID) of amino acids in foods[1,2]

Item	Toasted wheat bread	Whey protein isolate	Zein	Sorghum flour	Bovine collagen	Black beans	Pigeon peas	Chick peas	Roasted peanuts	All-Bran®	Pooled SEM	*P*-value
Indispensable amino acids, %												
Arg	104.3^{ab}	93.1^{bc}	72.2^{d}	108.2^{a}	94.7^{abc}	88.0^{c}	96.9^{abc}	98.8^{abc}	63.8^{d}	67.2^{d}	5.6	<0.05
His	96.0^{a}	93.4^{abc}	67.9^{d}	95.5^{ab}	85.4^{c}	69.7^{d}	85.7^{c}	87.5^{bc}	28.9^{f}	48.0^{e}	3.3	<0.05
Ile	95.5^{a}	93.5^{ab}	67.2^{d}	93.5^{ab}	90.9^{abc}	69.8^{d}	85.3^{bc}	84.3^{c}	11.9^{f}	41.9^{e}	3.3	<0.05
Leu	96.3^{a}	95.5^{a}	68.4^{c}	92.3^{ab}	87.6^{ab}	68.7^{c}	84.0^{b}	84.7^{b}	20.7^{e}	46.2^{d}	3.7	<0.05
Lys	85.1^{ab}	92.6^{a}	-472.0	91.9^{a}	84.0^{ab}	77.5^{b}	90.2^{a}	88.9^{ab}	-29.1^{d}	1.6^{c}	4.5	<0.05
Met	96.7^{a}	95.1^{a}	69.2^{c}	97.2^{a}	85.5^{ab}	68.0^{c}	82.2^{b}	88.1^{ab}	13.5^{e}	47.9^{d}	4.7	<0.05
Phe	96.8^{a}	93.5^{ab}	67.2^{c}	93.4^{ab}	88.9^{ab}	71.6^{c}	86.0^{b}	88.4^{ab}	31.3^{e}	51.6^{d}	3.4	<0.05
Thr	94.4^{a}	80.0^{bc}	69.6^{c}	91.5^{ab}	82.3^{abc}	56.3^{d}	79.9^{bc}	78.4^{c}	-38.3^{f}	12.2^{e}	4.5	<0.05
Trp	97.8^{a}	95.7^{a}	-55.6	97.3^{a}	85.8	65.1^{c}	78.0^{b}	85.9^{ab}	28.8^{e}	43.6^{d}	4.7	<0.05
Val	95.4^{a}	87.0^{ab}	69.0^{c}	92.8^{ab}	90.4^{ab}	60.1^{c}	82.9^{b}	81.8^{b}	-1.6^{e}	32.5^{d}	4.2	<0.05
Mean	96.4^{a}	91.2^{ab}	67.8^{c}	94.8^{ab}	90.3^{ab}	69.9^{c}	86.4^{b}	87.8^{ab}	24.5^{e}	41.5^{d}	3.5	<0.05

Table 5.7. (cont.)

Dispensable amino acids, %												
Ala	98.2^{a}	85.6^{bc}	67.8^{d}	95.0^{ab}	91.3^{abc}	60.3^{d}	81.9^{c}	84.9^{bc}	-4.9^{f}	32.4^{e}	4.6	<0.05
Asp	93.5^{a}	93.5^{a}	70.1^{cd}	95.5^{a}	62.5^{d}	61.9^{d}	85.8^{ab}	78.2^{bc}	27.5^{e}	5.6^{f}	3.7	<0.05
Cys	93.4^{a}	93.2^{a}	63.5^{b}	88.6^{a}	-37.6	8.3^{c}	64.2^{b}	62.4^{b}	-5.8^{d}	0.9^{cd}	5.1	<0.05
Glu	98.2^{a}	88.7^{b}	68.3^{c}	92.2^{ab}	86.4^{b}	74.0^{c}	89.0^{b}	87.6^{b}	43.1^{d}	70.4^{c}	2.9	<0.05
Gly	111.2^{ab}	83.6^{bc}	85.2^{bc}	128.7^{a}	77.8^{bc}	55.1^{c}	74.7^{bc}	87.8^{bc}	9.6^{d}	18.8^{d}	15.7	<0.05
Pro	132.5^{bc}	73.7^{c}	74.2^{c}	185.5^{ab}	90.0^{c}	189.5^{a}	168.3^{ab}	210.0^{a}	-33.1^{d}	84.9^{c}	31.3	<0.05
Ser	98.4^{a}	79.4^{cd}	71.7^{de}	93.9^{ab}	79.5^{cd}	64.9^{e}	82.9^{c}	86.5^{bc}	14.2^{g}	34.4^{f}	4.3	<0.05
Tyr	95.7^{a}	91.3^{ab}	68.0^{c}	90.8^{ab}	81.5^{b}	69.1^{c}	83.8^{b}	86.5^{ab}	38.0^{d}	42.1^{d}	3.7	<0.05
Mean	103.1^{ab}	84.8^{cd}	68.4^{e}	104.9^{a}	82.8^{cd}	73.3^{de}	88.8^{c}	92.6^{bc}	21.1^{g}	48.6^{f}	5.3	<0.05
Total amino acids	102.0^{a}	88.9^{b}	69.1^{c}	101.5^{a}	86.3^{b}	72.9^{c}	88.5^{b}	91.2^{ab}	23.9^{e}	47.2^{d}	4.3	<0.05

$^{a-g}$Means within a row lacking a common superscript letter differ ($P < 0.05$). Values lacking superscript were not included in

Table 5.7. (cont.)

Fisher's Least Significant Difference analysis or mean amino acids and total amino acids least squares means estimations.

[1]Data are least square means of 6 observations per treatment.

[2]Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses (g/kg of DMI) of amino acids were as follows: Arg, 1.01; His, 0.31; Ile, 0.57; Leu, 0.90; Lys, 0.91; Met, 0.16; Phe, 0.55; Thr, 0.86; Trp, 0.20; Val, 0.93; Ala, 1.01; Asp, 1.32; Cys, 0.29; Glu, 1.58; Gly, 2.57; Ser, 0.80; Tyr, 0.45.

Table 5.8. Digestible indispensable amino acid scores (DIAAS) values in experimental foods[1]

Ingredient	birth to 6 m[2]	6 m to 3 y[3]	3 y and older[4]
Toasted wheat bread	28 (Lys)	34 (Lys)	41 (Lys)
Whey protein isolate	61 (AAA)	82 (His)	103 (His)
Zein	0 (Lys)	0 (Lys)	0 (Lys)
Sorghum flour	31 (Lys)	37 (Lys)	45 (Lys)
Bovine collagen	0 (Trp)	0 (Trp)	0 (Trp)
Black beans	30 (SAA)	37 (SAA)	43 (SAA)
Pigeon peas	37 (Trp)	48 (SAA)	57 (SAA)
Chick peas	53 (Trp)	76 (SAA)	89 (SAA)
Roasted peanuts	0 (Thr)	0 (Thr)	0 (Thr)
All-Bran®	0 (Lys)	0 (Lys)	0 (Lys)

[1]First-limiting amino acid is in parentheses.

[2]DIAAS values were calculated using the recommended amino acid scoring pattern for an infant (birth to 6 months). The indispensable amino acid reference patterns are expressed as mg amino acid / g protein: His, 21; Ile, 55; Leu, 96; Lys, 69; sulfur amino acids, 33; aromatic amino acids, 94; Thr, 44; Trp, 17; Val, 55 (FAO, 2013).

Table 5.8. (cont.)

[3]DIAAS values were calculated using the recommended amino acid scoring pattern for a child (6m to 3y). The indispensable amino acid reference patterns are expressed as mg amino acid / g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; sulfur amino acids, 27; aromatic amino acids, 52; Thr, 31; Trp, 8.5; Val, 40 (FAO, 2013).

[4]DIAAS values were calculated using the recommended amino acid scoring pattern for older child, adolescent, and adult. The indispensable amino acid reference patterns are expressed as mg amino acid / g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; sulfur amino acids, 23; aromatic amino acids, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

Table 5.9. Intra-experiment variations in standardized ileal digestibility (SID) of amino acids in foods[1,2]

	Whey protein isolate				Sorghum flour				Pigeon peas			
Item	Group 1	Group 2	SEM	*P*-value	Group 1	Group 2	SEM	*P*-value	Group 1	Group 2	SEM	*P*-value
Indispensable amino acids, %												
Arg	93.1	104.8	9.0	0.202	108.2	109.0	2.8	0.346	96.9	97.9	1.0	0.503
His	93.4	95.7	1.8	0.242	95.5	91.6	2.6	0.337	85.7	86.2	2.2	0.698
Ile	93.5	94.3	0.6	0.359	93.5	88.7	3.6	0.310	85.3	86.4	2.0	0.602
Leu	95.5	96.5	1.0	0.316	92.3	88.7	2.9	0.372	84.0	86.5	2.4	0.355
Lys	92.6	94.8	1.4	0.175	91.9	88.3	8.5	0.819	90.2	91.4	1.6	0.472
Met	95.1	96.8	0.6	0.012	97.2	94.7	1.2	0.225	82.2	81.8	2.5	0.882
Phe	93.5	93.4	1.6	0.944	93.4	89.0	3.09	0.335	86.0	87.5	2.1	0.510
Thr	80.0	80.6	2.8	0.825	91.5	85.4	7.2	0.42	79.9	76.8	3.6	0.417
Trp	95.7	96.0	1.3	0.868	97.3	90.7	5.2	0.215	78.0	80.5	3.7	0.539
Val	87.0	88.5	2.04	0.480	92.8	88.03	5.4	0.404	82.9	83.0	2.6	0.986

Table 5.9. (cont.)

Mean	91.2	92.9	1.5	0.300	94.8	90.7	4.0	0.387	86.4	88.1	2.1	0.437
Dispensable amino acids, %												
Ala	85.6	89.1	2.8	0.231	95.0	91.6	3.0	0.423	81.9	85.0	2.7	0.308
Asp	93.5	95.2	1.4	0.213	95.5	91.3	4.3	0.288	85.8	87.4	1.7	0.576
Cys	93.2	93.9	1.4	0.646	88.6	84.8	4.1	0.445	64.2	59.2	4.6	0.308
Glu	88.7	91.4	1.4	0.082	92.2	89.2	2.6	0.438	89.0	90.4	1.3	0.308
Gly	83.6	116.9	21.5	0.158	128.7	109.8	10.6	0.143	74.7	80.8	12.7	0.763
Pro	73.7	131.9	38.1	0.189	185.5	193.3	7.5	0.760	168.3	228.7	37.2	0.188
Ser	79.4	83.3	3.9	0.363	93.9	91.4	4.6	0.628	82.9	86.6	3.9	0.420
Tyr	91.3	92.5	2.3	0.606	90.8	84.4	5.5	0.350	83.8	85.8	2.8	0.510
Mean	84.8	95.9	6.5	0.162	104.9	104.2	4.4	0.352	88.8	96.8	5.3	0.274
Total amino acids	88.9	95.4	3.9	0.144	101.5	99.8	4.1	0.326	88.5	93.8	3.5	0.294

[1]Data are least square means of 6 observations per treatment.

Table 5.9. (cont.)

[2]Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses (g/kg of DMI) of amino acids were as follows: Arg, 1.01; His, 0.31; Ile, 0.57; Leu, 0.90; Lys, 0.91; Met, 0.16; Phe, 0.55; Thr, 0.86; Trp, 0.20; Val, 0.93; Ala, 1.01; Asp, 1.32; Cys, 0.29; Glu, 1.58; Gly, 2.57; Ser, 0.80; Tyr, 0.45.

Table 5.10. Analysis of variation in digestible indispensable amino acid score (DIAAS) values within intra-experiment replication for selected proteins.

Item	Whey protein isolate	Sorghum flour	Pigeon peas
birth to 6 m[1]			
DIAAS, Group 1	61	31	37
DIAAS, Group 2	60	30	37
SEM	3.2	5.3	12.4
P-value	0.805	0.833	0.934
6 m to 3 y[2]			
DIAAS, Group 1	83	37	50
DIAAS, Group 2	82	36	48
SEM	3.1	5.2	9.4
P-value	0.241	0.873	0.377
3 y and older[3]			
DIAAS, Group 1	105	45	59
DIAAS, Group 2	102	43	56

Table 5.10. (cont.)

SEM	3.4	5.1	9.4
P-value	0.239	0.811	0.368

[1]DIAAS values were calculated using the recommended amino acid scoring pattern for an infant (birth to 6 months). The indispensable amino acid reference patterns are expressed as mg amino acid / g protein: His, 21; Ile, 55; Leu, 96; Lys, 69; sulfur amino acids, 33; aromatic amino acids, 94; Thr, 44; Trp, 17; Val, 55 (FAO, 2013).

[2]DIAAS values were calculated using the recommended amino acid scoring pattern for a child (6m to 3y). The indispensable amino acid reference patterns are expressed as mg amino acid / g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; sulfur amino acids, 27; aromatic amino acids, 52; Thr, 31; Trp, 8.5; Val, 40 (FAO, 2013).

[3]DIAAS values were calculated using the recommended amino acid scoring pattern for older child, adolescent, and adult. The indispensable amino acid reference patterns are expressed as mg amino acid / g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; sulfur amino acids, 23; aromatic amino acids, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

LITERATURE CITED

Abelilla, J. A., Y. Liu, and H. H. Stein. 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

AOAC Int. 2007. Official Methods of Analysis. 18th ed. Rev. 2. W. Howitz, and G. W. Latimer Jr., AOAC Int., Gaithersburg, MD.

Bindari, Y. R., H. N. Lærke, and J. V. Nørgaard. 2018. Standardized ileal digestibility and digestible indispensable amino acid score of porcine and bovine hydrolyzates in pigs. J. Sci. Food Agric. 98:2131-2137. doi:10.1002/jsfa.8697

Casas, G. A., and H. H. Stein. 2016. Effects of microbial xylanase on digestibility of dry matter, organic matter, neutral detergent fiber, and energy and the concentrations of digestible and metabolizable energy in rice coproducts fed to weanling pigs. J. Anim. Sci. 94:1933-1939. doi:10.2527/jas.2015-0064

Cervantes-Pahm, S. K., Y. Liu, and H. H. Stein. 2014. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br. J. Nutr. 111:1663-1672. doi:10.1017/s0007114513004273

Di Lullo, G. A., S. M. Sweeney, J. Körkkö, L, Ala-Kokko, and J. D. S. Antonio. 2002. Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen. J. Biol. Chem. 277:4223-4231. doi:10.1074/jbc.M110709200

Fabbri, A. D. T., and G. A. Crosby. 2015. A review of the impact of preparation and cooking on the nutritional quality of vegetables and legumes. Int. J. Gastron. Food. Sci. 3:2-11. doi:10.1016/j.ijgfs.2015.11.001

FAO. 1991. Protein quality evaluation. Report of a joint FAO/WHO expert consultation. FAO food and nutrition paper 51. Food and Agriculture Organization of the United Nations. Bethesda, 1991.

FAO. 1999. Phaseolus bean: post-harvest operations. Centro Internacional de Agricultura Tropical. Food and Agriculture Organization of the United Nations. Rome, 1999.

FAO. 1999b. Sorghum: post-harvest operations. Natural Resources Institute. Food and Agriculture Organization of the United Nations. Rome, 1999.

FAO. 1999c. Wheat: post-harvest operations. Pakistan Agricultural Research Council. Food and Agriculture Organization of the United Nations. Rome, 1999.

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. FAO food and nutrition paper 92. Food and Agriculture Organization of the United Nations. Rome, 2013.

FAOSTAT. 2014. Wheat - the largest primary commodity. Food and Agriculture Organization of the United Nations. Rome, 2013.

FAO. 2018. Food outlook biannual report on global food markets. Food and Agriculture Organization of the United Nations. Rome, 1999.

Foyer, C. H., H.-M. Lam, H. T. Nguyen, K. H. M. Siddique, R. K. Varshney, T. D. Colmer, W. Cowling, H. Bramley, T. A. Mori, J. M. Hodgson, J. W. Cooper, A. J. Miller, K. Kunert, J. Vorster, C. Cullis, J. A. Ozga, M. L. Wahlquist, Y. Liang, H. Shou, K. Shi, J. Yu, N. Fodor, B. N. Kaiser, F.-L. Wong, B. Valliyodan, and M. J. Considine. 2016. Neglecting legumes has compromised human health and sustainable food production. Nat. Plants. 2:16112. doi:10.1038/nplants.2016.112

Izquierdo, P., C. Astudillo, M. W. Blair, A. M. Iqbal, B. Raatz, and K. A. Cichy. 2018. Meta-QTL analysis of seed iron and zinc concentration and content in common bean (Phaseolus vulgaris L.). Theor. Appl. Genet. 131: 1645. doi:10.1007/s00122-018-3104-8

Joshi, P. K. and P. P. Rao. 2017. Global pulses scenario: status and outlook. Ann. N. Y. Acad. Sci. 1392:6-17. doi:10.1111/nyas.13298

Liu, Y., M. Song, F. N. Almeida, S. L. Tilton, M. J. Cecava, and H. H. Stein. 2014. Energy concentration and amino acid digestibility in corn and corn coproducts from the wet-milling industry fed to growing pigs. J. Anim. Sci. 92:4557-4565. doi:10.2527/jas.2014-6747

Liu, Y., and P. K. W. Ng. 2014. Isolation and characterization of wheat bran starch and endosperm starch of selected soft wheats grown in Michigan and comparison of their physicochemical properties. Food Chem. 176:176-144. doi:10.1016/j.foodchem.2014.12.023

Mathai, J. K., Y. Liu, and H. H. Stein. 2017. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). Br. J. Nutr. 117:490-499. doi:10.1017/S0007114517000125

Marinangeli, C. P. F., J. Curran, S. I. Barr, J. Slavin, S. Puri, S. Swaminathan, L. Tapsell, and Patterson, C. A. 2017. Enhancing nutrition with pulses: defining a recommended serving size for adults. Nutr. Rev. 75:990–1006. doi:10.1093/nutrit/nux058

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82:179-183. doi:10.2527/2004.821179x

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC. doi:10.17226/13298

Ofuya, Z. M., and V. Akhidue. 2005. The role of pulses in human nutrition: a review. J. Appl. Sci. Environ. Mgt. 9:99-104. doi:10.4314/jasem.v9i3.17361

Reddy N. R., M. D. Pierson, S. K. Sathe, D. K. Salunkhe. 1985. Dry bean tannins: a review of nutritional implications. J. Am. Oil Chem.' Soc. 62:541-549. doi:10.1007/BF02542329

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

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

Saxena, K. B., R. V. Kumar, and C. L. L. Gowda. 2010a. Vegetable pigeonpea – a review. J. Food. Leg. 23:91-98. doi:10.1111/j.1365-2621.1984.tb12489.x

Saxena, K. B., R. V. Kumar, and R. Sultana. 2010b. Quality nutrition through pigeonpea – a review. J. Food. Leg. 2:1335-1344. doi:10.4236/health.2010.211199

Schaafsma, G. 2000. The protein digestibility-corrected amino acid score. J. Nutr. 130:1865S-1867S. doi:10.1093/jn/130.7.1865S

Shukla, R. and M. Cheryan. 2001. Zein: the industrial protein from corn. Ind Crops Prod. 13:171-192. doi: 10.1016/S0926-6690(00)00064-9

Snapp, S. S., R. B. Jones, E. M. Minja, J. Rusike, and S. N. Silim. 2003. Pigeon pea for Africa: a versatile vegetable – and more. Hort. Sci. 38:1073-1079.

Song, H., S. Zhang, L. Zhang, B. Li. 2017. Effect of orally administered collagen peptides from bovine bone on skin aging in chronologically aged mice. Nutrients. 9:1209-1223. doi: 10.3390/nu9111209

Stein, H. H., C. F. Shipley, and R. A. Easter. 1998. Technical note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1443-1436. doi:10.2527/1998.7651433x

Stein, H. H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. de Lange. 2007. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282-285. doi:10.1016/j.livsci.2007.01.019

Urriola, P. E., S. K. Cervantes-Pahm, and H. H. Stein. 2013. Fiber in swine nutrition. In: L. I. Chiba, ed. Sustainable Swine Nutrition. 1st ed. John Wiley & Sons, Inc., Ames, IA. p. 255-276.

CHAPTER 6: CONCLUDING REMARKS

Protein is an essential component of the human diet a source of indispensable amino acids. Yet, throughout the developing world, amino acid deficiency is rampant. Although amino acid deficiency incidence rates are multi-factorial, one of the primary causes is the consumption of staple foods that are low in indispensable amino acids. Changing dietary patterns in the developing world would require great efforts and large cultural shifts. However, small alterations to the diet made to optimize amino acid profiles using data from protein quality assessments may have lasting effects. Therefore, it is critical that a variety of foods are evaluated for their protein quality and a large number of DIAAS values are made available for use.

Protein quality evaluation is not a new science. Proteins have long been evaluated for their capacity to serve as a nutrient source in diets. Early on in protein nutrition research, it was clear that not all proteins were equal. However, the shift in viewing a protein's amino acids as individual nutrients is relatively recent. Nonetheless, this change in perspective has been widely adopted and hundreds of proteins have been evaluated based on their amino acid compositions. Yet, as our understanding of amino acid nutrition increased, considerations for bioavailability became of greater concern. One of the first systems for evaluating protein value as a nutrient based on both its amino acid composition and an estimate for bioavailability was the protein digestibility corrected amino acid scoring (**PDCAAS**) system. This system was a significant improvement over simple amino acid profiles, and as a result PDCAAS was considered the gold-standard for protein quality research for over 25 years.

Despite PDCAAS' prevalence and proven value to the nutritionist, it became clear that improvements to it utilizing modern advancements could be made. In particular, a more apt

animal model and better estimates for digestibility of amino acids were now available. Utilizing these updates, the digestible indispensable amino acid scoring (**DIAAS**) was proposed. Two fundamental issues prevent the immediate adoption of DIAAS as the standard for protein quality evaluation: 1) a lack of evidence that the DIAAS system is an accurate, precise, and consistent method for protein quality evaluation and, 2) the lack of a large database of DIAAS values for foods.

The overall objective of this research was to utilize the DIAAS system to determine protein quality values for various foods, while simultaneously assessing its merit as a methodology worthy of replacement of the PDCAAS system. Combined, the DIAAS values for over 20 different proteins were determined in these experiments. Many of these proteins represent staple foods of large segments of the population in the developing world. The DIAAS values generated for those proteins highlight the relative deficiency in indispensable amino acids that those populations are subject to, but also offer valuable information on how to best combat those deficiencies with complementary proteins. Various high quality proteins were evaluated in these experiments, many of which represent potential options for use as complementary proteins in the developing world.

In addition to the generation of DIAAS values, this research also helped to compare the DIAAS system to the current PDCAAS system and demonstrated that PDCAAS values overestimate the protein quality of certain foods. Although, perhaps of seemingly low consequence, the implications of overestimating protein quality, particularly with respect to populations at risk of amino acid deficiency, are dire. This research also helped to validate the DIAAS system as a robust and repeatable methodology for evaluating various protein sources.

As a result, this research has strengthened the argument that the DIAAS system is not only an acceptable model for protein quality evaluation, but also the preferred.