ADDITIVITY OF VALUES FOR DIGESTIBLE INDISPENSABLE AMINO ACID SCORE IN INDIVIDUAL FOODS WHEN INCLUDED IN MIXED MEALS FOR HUMANS

BY

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THESIS

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ABSTRACT

Two experiments were conducted to determine protein quality in animal- and plant-based food ingredients using the digestible indispensable amino acid score (DIAAS) method and to test the hypothesis that animal proteins can complement low-quality proteins and that values for DIAAS are additive in mixed meals. In experiment 1, three diets contained a breakfast cereal (i.e., cornflakes or quick oats) or dry milk as the sole source of amino acids (AA). Two additional diets contained a combination of dry milk and cornflakes or quick oats. A nitrogen-free diet was also used. Six ileal cannulated gilts [average body weight: 85.1 ± 7.7 (SD) kg] were allotted to a 6 × 6 Latin square design with six diets and six 7-day periods. The first 5 days were considered the adaptation period to the diet and ileal digesta were collected for 9 h on days 6 and 7 of each period. Values for standardized ileal digestibility (SID) and DIAAS were calculated for cornflakes, quick oats, dry milk, and the two combined meals for children from 6 months to 3 years old and for individuals older than 3 years of age. For the combined meals, SID and DIAAS were also predicted from the individual ingredient values. For both age groups, dry milk had greater ($P < 0.05$) DIAAS (123 and 144 for children and older individuals, respectively) than quick oats (57 and 67 for children and older individuals, respectively), and cornflakes had lower ($P < 0.05$) DIAAS (16 and 19 for children and older individuals, respectively) than the other ingredients. Both breakfast cereal-dry milk meals had DIAAS close to or greater than 100 for children aged 6 months to 3 years and for older individuals, but there were no differences between measured and predicted DIAAS. Results indicated that the combination of milk and breakfast cereals provided a meal that is balanced in indispensable AA for humans, and based on SID, DIAAS obtained from individual ingredients is additive in mixed meals. In experiment 2, SID and DIAAS were determined using the same procedures as in experiment 1. Six diets contained a burger patty (i.e., 80% lean beef, 93% lean beef, 80% lean pork, Impossible Burger, or Beyond Burger) or a burger bun as the sole source of AA. Three additional diets were based on a combination of the bun and a patty from 80% beef, pork, or Impossible Burger. A nitrogen-free diet was also used. Ten ileal cannulated gilts [average body weight: 24.6 ± 1.3 (SD) kg] were allotted to a 10 × 6 Youden square design with ten diets and six 9-day periods, with ileal digesta being collected on days 8 and 9 of each period. For both age groups, the DIAAS values for 93% lean beef and pork burgers (111 and 119 for children and older individuals, respectively) were greater ($P < 0.05$) than the plant-based burgers (Impossible Burger: 91 and 107 for children and older individuals, respectively; Beyond Burger: 71 and 83 for children and older individuals, respectively). The 80% lean beef burger had greater ($P < 0.05$) DIAAS (102 and 110 for children and older individuals, respectively) than the plant burgers for children from 6 months to 3 years, but greater ($P < 0.05$) DIAAS than the Beyond Burger for individuals older than 3 years. The burger bun had the lowest ($P < 0.05$) DIAAS values (26 and 31 for children and older individuals, respectively) among all ingredients for both age groups. There were no differences between the measured and predicted DIAAS. Results indicated that the protein quality of animal-based burgers is greater than that of plant-based burgers. However, for individuals older than 3 years, the Impossible Burger has protein quality comparable to that of the 80% lean beef burger. Based on SID, the DIAAS obtained from individual foods were also additive in mixed meals. In conclusion, animal-based ingredients had DIAAS close to or greater than 100 and can be used to complement plant-based proteins. In addition, DIAAS for individual ingredients were additive and can be used to calculate DIAAS in mixed meals, and this is important because it is not possible to measure DIAAS for all types of food combinations.

Key words: Animal proteins, amino acids, digestible indispensable amino acid score, plant proteins, protein quality.

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CHAPTER 1: INTRODUCTION

Protein quality is an important aspect of human nutrition and is influenced by several factors such as amino acid (**AA**) composition, digestibility, and bioavailability of a protein source (Schönfeldt and Hall, 2012). The most widely used method for evaluating protein quality is the protein digestibility corrected amino acid score (**PDCAAS**), which was developed by the Food and Agriculture Organization of the United Nations (**FAO**) and the World Health Organization (**WHO**) in 1991 (FAO/WHO, 1991). However, the PDCAAS method has been criticized for its limitations, particularly its inability to take into account the individual AA requirements of humans and the digestibility of each AA of the protein source (Schaafsma, 2012; Mathai et al., 2017). As a result, a more recent method called the digestible indispensable amino acid score (**DIAAS**) was developed to assess protein quality by taking into account the AA concentration, digestibility, and bioavailability of the foods (FAO, 2013).

The DIAAS method determines the amount of digestible indispensable amino acids (**DIAA**) in relation to human requirements, which is considered a more accurate way of determining protein quality, and has become a useful tool for evaluating the suitability of different protein sources for human consumption, as well as determining the protein requirements for specific populations (Millward, 2012). When a human model is not available, FAO experts recognized that evaluating protein quality using a pig model was preferable, and that ileal AA digestibility better describes protein quality than fecal crude protein (**CP**) digestibility because digestibility determined at the terminal ileum best reflects the amount of absorbed AA (FAO, 2013; Mathai et al., 2017). Furthermore, the DIAAS method allows for calculation of the protein value of a meal consisting of different proteins, however, additivity of DIAAS in combined meals must be assessed (FAO, 2014).

A database on AA digestibility and DIAAS from different foods must be established before this method can be widely used, particularly by regulatory agencies concerned with protein quality (FAO, 2014). Nevertheless, several DIAAS values in plant- and animal-based proteins such as grains, pulses, dairy, and meats, have been determined (Cervantes-Pahm, 2014; Mathai et al., 2017; Abelilla et al., 2018; Hodgkinson et al., 2018; Han et al., 2019; Bailey et al., 2020). However, data demonstrating the ability of higher quality proteins to complement lower quality protein ingredients to produce a meal that is adequate in digestible AA are limited, and there is no data demonstrating DIAAS additivity in mixed meals. Therefore, the objectives of this thesis were to 1) determine AA digestibility and DIAAS values in cereal grains, dairy, and meat products, and test the hypothesis that high-quality animal-based proteins can be used to supplement low-quality plant-based proteins, and 2) test the hypothesis that DIAAS measured in a mixed meal is not different from the DIAAS calculated from the individual ingredients in the meal.

Literature Cited

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

Bailey, H. M., Mathai, J. K., Berg, E. P., and Stein, H. H. 2020. Pork products have digestible indispensable amino acid scores (DIAAS) that are greater than 100 when determined in pigs, but processing does not always increase DIAAS. J. Nutr. 150:475-482. doi:10.1093/jn/nxz284

Cervantes-Pahm, S. K., Liu, Y., and Stein, H. H. 2014. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br. J. Nutr. 111:1663-1672. doi:10.1017/S0007114513004273

FAO/WHO. 1991. Protein quality evaluation. Report of the joint FAO/WHO expert consultation. FAO food and nutrition paper 51. Bethesda, MD.

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. Rome.

FAO. 2014. Research approaches and methods for evaluating the protein quality of human foods. Report of an FAO expert working group. Rome.

Han, F., Han, F., Wang, Y., Fan, L., Song, G., Chen, X., Jiang, P., Miao, H., and Han, Y. 2019. Digestible indispensable amino acid scores of nine cooked cereal grains. Br. J. Nutr. 121:30-41. doi:10.1017/S0007114518003033

Hodgkinson, S. M., Montoya, C. A., Scholten, P. T., Rutherfurd, S. M., and Moughan, P. J. 2018. Cooking conditions affect the true ileal digestible amino acid content and digestible indispensable amino acid score (DIAAS) of bovine meat as determined in pigs. J. Nutr. 148:1-6. doi:10.1093/jn/nxy153

Mathai, J. K., Liu, Y., and Stein, H. H. 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

Millward, D. J. 2012. Amino acid scoring patterns for protein quality assessment. Br. J. Nutr. 108:S31-S43. doi:10.1017/S0007114512002462

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

CHAPTER 2: PROTEIN QUALITY AND ADDITIVITY OF VALUES FOR PROTEIN SCORES: LITERATURE REVIEW

Introduction to Protein Nutrition

Protein is an essential component of human nutrition and plays an important role in many physiological processes including growth and repair of tissues, hormone regulation, and immune function. Adequate protein intake is crucial for maintaining good health and preventing chronic diseases (Millward et al., 2008). The recommended protein intake for adults is 0.8 grams per kilogram of body weight per day (FAO/WHO/UNU, 2007). However, not all proteins are equal because they may have different concentrations of dispensable and indispensable amino acids (**AA**). Because the human body requires indispensable AA rather than protein, it is the concentration of indispensable AA in a protein that determines its quality. In general, animal proteins (milk, eggs, meat, fish) contain more indispensable AA per unit of protein than plant proteins, and among plant proteins, oilseeds and oilseed meals have greater quality than proteins from pulses or cereal grains. Although most Americans have daily intake of protein above the requirement, there are groups of people who either do not eat enough protein or consume protein of low quality (i.e., with low concentration of indispensable AA). As a result, certain populations, including children, athletes, pregnant women, and the elderly, may suffer from undernutrition because of low AA intake (Volpi et al., 2003; Elango et al., 2010; Witard et al., 2019). In addition, due to poverty, it is estimated that 22% of children under the age of five worldwide consume indispensable AA at quantities below requirements (UNICEF/WHO/World Bank Group, 2021).

Undernutrition, specifically protein-energy malnutrition is a major global health issue that affects millions of people, primarily in developing countries, and it is characterized by a lack of both protein and energy intake and can have serious consequences for physical and cognitive development, as well as overall health, including stunted growth, decreased muscle mass, weakened immunity, and increased susceptibility to infections (Batool et al., 2015). Undernutrition can lead to the development of Marasmus (long-term inadequate intake of macronutrients and micronutrients) and Kwashiorkor (lack of protein in the diet despite sufficient calorie intake), which are severe forms of malnutrition that primarily affect children in developing countries (Lelijveld et al., 2016). Undernutrition is also influenced by poverty, food insecurity, and poor sanitation (Domènech, 2015). As of 2021, the global rate of undernourishment was around 9.8%, and the number of people unable to afford a healthy diet increased, reflecting the impact of rising food prices during the COVID-19 pandemic (WFP/WHO/UNICEF, 2022).

Protein consumption and demand are influenced by a variety of factors, but as income increases, so does protein consumption (Aggarwal and Drewnowski, 2019). Individuals with higher incomes and education consume more protein than those with lower incomes and education, and higher income is also associated with greater consumption of animal-based protein sources. In contrast, consumption of mostly lower-quality plant-based proteins including legumes, grains, nuts, and seeds are more common by lower-income individuals (Mayen et al., 2014). Low dietary intake of other indispensable nutrients is also linked to low socioeconomic individuals or individuals residing in rural areas, resulting in deficiencies, not only in indispensable AA, but also in minerals and vitamins (Mayen et al., 2014). As a result, strategies and policies to prevent or manage nutrient-related disorders must be adopted to address availability and affordability (WFP/WHO/UNICEF, 2022).

Although animal proteins have greater AA quality than plant proteins, increased consumption of animal proteins will increase diet costs when compared with consumption of plant proteins due to the greater cost (Aggarwal and Drewnowski, 2019). The Economic Research Service of the USDA estimated that livestock prices increased 17% in 2021 compared with the previous year, and reached the greatest prices since 2015 (ERS/USDA, 2023). In contrast, despite the fact that plant protein prices also have increased due to global population expansion and biofuel production, inflation-adjusted prices for plant proteins have remained steady or decreased compared with previous years, and the gap between prices of plant and animal proteins has, therefore, increased in recent years (ERS/USDA, 2019). However, due to the differences in quality between animal and plant proteins, price comparisons cannot be made based on total protein concentrations. There is, therefore, a need to establish procedures that estimate the quality of proteins.

Protein Quality

The concept of protein quality and the need to evaluate the quality of protein sources have been a topic of interest in human nutrition for more than a century. Indispensable AA concentration and composition, digestibility, and bioavailability are important in determining protein quality (FAO/WHO, 1991). The first method for evaluating protein quality, the protein efficiency ratio (**PER**), was introduced in the early 20th century and measures the weight gain of laboratory animals (typically rats) fed a specific protein source (Hoffman and Falvo, 2004). Later, the biological value (**BV**) of protein was also introduced to evaluate protein quality by measuring the proportion of absorbed nitrogen that was retained in the body (Mitchell, 1924; Hoffman and Falvo, 2004). In 1991, the protein digestibility corrected amino acid score (**PDCAAS**) method was developed by the Food and Agriculture Organization of the United Nations (**FAO**) and the World Health Organization (**WHO**) by expressing the digestible content of the first limiting essential AA of a test protein as a percentage of the concentration of the same AA in a reference AA pattern (FAO/WHO, 1991). In 2011, FAO proposed that the digestible indispensable amino acid score (**DIAAS**) is a more accurate method for determining protein quality compared with traditional methods. In the DIAAS procedure, the ratio between the ileal digestible concentration of each AA of the protein source and individual AA requirements of humans is calculated (FAO, 2013). Each of the mentioned procedures have advantages and disadvantages, but they all attempt to provide a measure of quality assessment to each protein.

Protein efficiency ratio (PER) method

The PER method was developed in response to a publication that reviewed a method for numerically expressing the growth-promoting value of proteins, because it was understood that protein is defined not only by its absolute amount, but also by its quality (Osborne et al., 1919). The method was established under certain standardized conditions and it was based on the rate of growth of weanling rats, which was measured using the following equation (Lamb and Harden, 1973):

$$\text{PER} = \text{weight gain of the rat (g)} / \text{protein consumed by the rat (g)}$$

Rats were fed a test protein for a specific time (usually 28 days) as well as a casein protein control diet and the weight gain was measured. The standard value for casein is 2.5, and the value for the tested protein was compared with the casein value to determine protein quality.

If the value exceeded 2.5, the protein ingredient was considered an excellent protein source (Mansilla et al., 2020).

The PER method has the advantage of being simple to implement and conduct, and the PER of a food is also reasonably simple to generate. However, limitations of this method have been identified (Lamb and Harden, 1973). Because diets may differ in their ability to support growth of specific tissues, weight gained as fat and as lean body mass cannot be differentiated and may vary according to the protein source and level (Lamb and Harden, 1973). Flaws associated with differences in AA requirements between rats and humans are also acknowledged. As an example, rats have substantially greater sulfur amino acid (**SAA**) requirements than humans, which can result in a lower PER (NRC, 1995). Nevertheless, despite the fact that PER has many limitations, the method is still being used as the official method to evaluate protein quality of infant formulas in the U.S. and until recently, it was used as the general measure of protein quality in Canada (FDA, 2018).

The PER can also be influenced by other factors, such as the age and weight of the rats used in the assay, the feeding period, and the composition of the diet. As a result, advancements were achieved over time, particularly in maintaining a consistent protein level in all diets (Mansilla et al., 2020). Modifications to the PER method were proposed, and the net protein ratio (**NPR**) approach was developed to reduce the experimental time from 28 days to 14 days and to estimate maintenance requirement using a nitrogen-free diet and comparing with rats fed the test proteins (Pellett and Young, 1980):

NPR = (weight gain – weight gain of the nitrogen-free group) / (protein consumed – protein consumed by the nitrogen-free group)

Although NPR is more advantageous than PER, it has a limited approach and provides a single ratio that may oversimplify the complex nature of protein utilization in the body. Therefore, the relative net protein ratio (**RNPR**) procedure was later developed to address shortcomings and calculate the ratio of a protein NPR and a reference protein NPR to increase the precision of calculated values (Bodwell et al., 1989). Although the RNPR was considered an improvement to the PER method, it was determined that another method based on a comparison of the AA content needed to be established (FAO, 2013).

Biological value (BV) of protein method

The BV technique was first described by Karl Thomas as the protein digestion products that the body uses to meet the metabolic demand of AA for protein synthesis, and it was further explored in rats by assessing the metabolic nitrogen of the feces and urine using a nitrogen-free diet (Mitchell, 1924). This method is based on nitrogen balance rather than measuring growing animal parameters for evaluating protein quality, and included direct determinations of nitrogen in feces and urine, as well as indirect determinations of the fractions of fecal and urinary nitrogen that were derived from the diet. The BV of a protein was then determined using the following equation (Mitchell, 1924):

BV (%) = {[(nitrogen intake – nitrogen output in feces and urine) – (nitrogen intake of nitrogen-free diet – nitrogen output in feces and urine from nitrogen-free diet)] / [nitrogen intake – (nitrogen output in feces – nitrogen output in feces from nitrogen-free diet)]} × 100

The best that can be achieved for any amount of absorbed nitrogen is that the AA pattern exactly matches the requirements, ensuring that all AA are being utilized (Mitchell, 1924; FAO, 2013). However, the BV method implies that scores cannot exceed 100 because metabolic demand requires both dispensable and indispensable AA, and if AA are in excess of the requirement, some of the absorbed AA would not be utilized for protein synthesis (FAO, 2013). In a modification of the BV method, the net protein utilization (**NPU**) method was developed to reflect not only nitrogen absorption, but also nitrogen utilization in terms of nitrogen intake, using the following formula (Friedman, 1996):

NPU (%) = {[nitrogen intake – (nitrogen in feces – nitrogen in feces from the nitrogen-free diet] / nitrogen intake} × BV

However, both methods have been criticized for not taking into account the individual AA requirements of humans (FAO, 2013).

Protein digestibility corrected amino acid score (PDCAAS) method

The PDCAAS method is based on the concept of limiting AA compared with a reference human AA requirement pattern corrected for the digestibility of the protein (FAO, 1991). One of the advantages of the PDCAAS method is that it takes into account both the AA composition of the protein and its digestibility, which makes it a more accurate method for evaluating protein quality compared with earlier methods such as PER and BV (FAO, 1991; FAO, 2013). The PDCAAS method evaluates the protein quality in foods using rat experiments. Rats are fed diets containing a certain amount of protein as well as a nitrogen-free diet. Feces are then collected to measure the standardized total tract digestibility (**STTD**) of nitrogen (Kong and Adeola, 2014). The fecal nitrogen detected in the nitrogen-free diet is considered the basal endogenous loss of nitrogen secretions representing the metabolic nitrogen (FAO, 1991). Values for STTD of nitrogen is multiplied by the concentration of each AA in the food and this value is divided by the concentration of crude protein (**CP**) in the food to calculate the digestible AA per gram of protein (Mathai et al., 2017). The digestible AA per gram of protein is divided by the AA reference pattern for preschool children from 2 to 5 years old (FAO, 1991). The PDCAAS is then calculated using the following equation (Schaafsma, 2000):

PDCAAS (%) = lowest digestible AA ratio × 100

Although the PDCAAS approach has being used for more than 30 years to evaluate protein quality, flaws of this method have been recognized (Schaafsma, 2000; FAO, 2013; Mathai et al., 2017). The PDCAAS method does not adequately take into account the bioavailability of individual AA (FAO, 2013). It is recognized that digestibility of AA is more accurately measured at the end of the small intestine, and that nitrogen measured in the feces may have been influenced by hindgut fermentation (Sauer and Ozimek, 1986; Stein et al., 2007). Therefore, the assumption that all AA have the same digestibility, and that total tract nitrogen digestibility is representative of AA digestibility is incorrect because each AA are digested with different efficiencies (Stein et al., 2007; Mathai et al., 2017).

The PDCAAS method also does not recognize the correct nutritional value of high-quality proteins because values are truncated to 100 with the rationale that any AA exceeding the requirement confers no additional benefits. However, this is only true if the protein is fed alone, but if a combination of proteins are consumed, high quality proteins may complement low quality proteins and provide a balanced meal (Schaafsma, 2000; FAO, 2013). Another limitation of the PDCAAS approach is the use of one scoring pattern that is based on AA requirements for preschool children from 2 to 5 years of age, and therefore, does not take into account individual needs of different populations such as infants, older children, adolescents, or adults (Mathai et al., 2017). In addition, because the total tract digestibility of nitrogen is assumed to be representative of all AA, PDCAAS overestimates the protein quality of foods low in digestible

AA and foods containing antinutritional factors. In contrast, PDCAAS underestimates the value of high-quality protein sources (FAO, 2013; Rutherfurd et al., 2014).

Some flaws of the PDCAAS method may be overcome if different scoring patterns are used, and if values are not truncated to 100 (Mathai et al., 2017). However, the fact that the STTD of CP is used to calculate PDCAAS becomes a significant limitation in predicting the ileal digestibility of each AA, and further recommendations to overcome this were published (FAO, 2013). Nevertheless, despite limitations, PDCAAS is still widely used to evaluate protein quality and for regulatory purposes, and it has helped identify high-quality protein sources and guide food fortification and supplementation programs.

Digestible indispensable amino acid score (DIAAS) method

To overcome some of the limitations with the PDCAAS method, the DIAAS procedure was proposed (FAO, 2013). This method assesses protein quality using standardized ileal digestibility (**SID**) to account for absorption and utilization of AA rather than STTD of nitrogen (FAO, 2013). Ideally, values for SID should be determined in humans, but collecting digesta from humans can be expensive and invasive, making it unsuitable for routine evaluation of proteins (FAO, 2013; Hodgkinson et al., 2020). The growing pig was, therefore, recognized as the best model to determine SID of AA, but if pigs are not available, the growing laboratory rat can be used (FAO, 2013).

Values for the SID of AA in some foods measured in growing pigs are available, and these data can be easily used to calculate DIAAS (Hodgkinson et al., 2020). However, despite the similarities of the animal model with humans, absorption and digestibility are unlikely to be identical, and environmental or social factors are not accounted for (FAO, 2014). Measuring digestibility in ileal digesta also requires more resources than measuring digestibility in feces, and the DIAAS method requires detailed information about the digestibility of the protein source, which is not always readily available (FAO, 2013).

Procedures for determining DIAAS in food proteins are based on recommended guidelines from FAO. Growing female pigs from 30 to 100 kg body weight with a T-cannula inserted in the distal ileum should be used (Stein et al., 1998; FAO, 2014). Pigs should be fed a minimum of 5 days per test diet and protein sources must be provided in the same form as they are consumed by humans. Daily meals need to be divided into two meals fed 9 hours apart (FAO, 2014; Hodgkinson et al., 2020). A nitrogen-free diet with purified corn starch should also be fed to measure basal endogenous losses of AA and titanium dioxide should be used as an ingestible marker. Ileal digesta is then collected over two periods of at least 9 hours and frozen as soon as possible (FAO, 2014).

In the DIAAS method, each indispensable AA is considered a separate nutrient, and the difference between PDCAAS and DIAAS is that the SID of each AA is used. Therefore, the concentration of SID in each AA is calculated by multiplying the SID (%) by the concentration of that AA in the food ingredient, and this value is then divided by the concentration of CP to obtain the digestible indispensable amino acid (**DIAA**) content per protein.

Values for DIAAS are calculated using the DIAA reference ratios (FAO, 2013):

DIAA reference ratio = mg of DIAA content in 1 g protein of food / mg of the same DIAA in 1g of the reference protein

DIAAS (%) = lowest value of DIAA reference ratio × 100

Claims based on DIAAS values can be made to show protein quality in a more practical way. Values from 75 to 99 are considered "good sources" of protein, values equal to or greater than 100 are considered "excellent sources" of protein, but no claims can be made for proteins with a score of less than 75 (FAO, 2013). Separate ratios are calculated for infants (between the ages of 0 and 6 months), children (between the ages of 6 months and 3 years), and older children, adolescents, and adults (older than 3 years of age), demonstrating that DIAAS has different scoring patterns compared with PDCAAS and takes into account the AA requirement of different age groups (FAO, 2013). In addition, values for DIAAS are not truncated to 100, allowing the calculation of not only the protein source, but also for meals containing different proteins. As a consequence, if values for DIAAS obtained in individual ingredients are additive in mixed meals, DIAAS in mixed meals or foods prepared with multiple ingredients can be calculated (FAO, 2014).

The advantage of using DIAAS is that it takes into account the individual AA requirements of humans, and it not only demonstrates the complementary effects of higher-quality proteins, but it also assesses the adequacy of protein consumption in individuals consuming lower-quality proteins (Mathai et al., 2017). However, to calculate DIAAS and establish this approach for protein quality evaluation, there is a need to construct a fully accessible database with values for AA digestibility of foods from various parts of the world (FAO, 2013; Hodgkinson et al., 2020). In addition, more inter-species comparisons, food processing methods, protocols for vulnerable populations, including teenagers, lactating women, athletes, and elderly with new scoring patterns, and evaluations on the practical impacts of public health policies are needed before DIAAS can be used for regulatory purposes (FAO, 2014).

Published Data for DIAAS: Overview

Values for DIAAS have been determined for several food items, and although differences in DIAAS values measured in *in vivo* experiments using rats or pigs may occur due to species requirements or methodologies used, it is important to acknowledge published data for DIAAS of different foods to allow the implementation of this method in diet formulations (FAO, 2014).

Animal-based proteins

The highest DIAAS values are, in general, obtained in animal-based proteins such as dairy, eggs, meat, and fish (Table 2.1). Animal-based proteins generally have a greater protein quality than plant-based proteins because of a better balance among the nine indispensable AA compared with plant-based proteins that are usually deficient in one or more of these AA (Wu, 2016). This AA balance is essential for synthesizing body proteins, including muscle protein (Phillips, 2017). Animal-based proteins are also generally more digestible, meaning that a greater percentage of the AA consumed is absorbed and available for protein synthesis in the body (Rutherfurd et al., 2014). Processing is also one of the factors that contribute to the greater protein quality in animal-based proteins compared with plant-based proteins because most animal proteins are less processed than plant proteins and processing can reduce AA concentration and bioavailability (Finot, 1981).

Plant-based proteins

Values for DIAAS in several plant-based proteins including grains, pulse crops, and nuts have been determined (Table 2.2). Whereas these proteins are often low in digestible AA, some plant-based proteins such as soy and soy products have greater DIAAS compared with other grains (Mathai et al., 2017). Although plant-based proteins present health benefits including low saturated fat and greater fiber than animal-based proteins, they usually have an incomplete AA profile and lack some of the indispensable AA (Wu, 2016). However, it is possible to combine different plant-based protein sources to create a more balanced diet (Herreman et al., 2020). Plant proteins also often contain anti-nutritional factors such as phytic acid and lectins that can interfere with the absorption of nutrients, thereby reducing digestibility of AA (Schlemmer et al., 2009). Therefore, digestibility of plant-based proteins is usually less compared with most animal-based proteins because of anti-nutrients, protein structure, and processing or cooking methods that are used to produce human foods (Mariotti and Gardner, 2019). Despite these factors, individuals strictly consuming a plant-based diet may be able to consume all indispensable AA to meet AA requirements by consuming a variety of plant-based proteins that are selected to complement each other in terms of AA composition to ensure adequate protein intake (Herreman et al., 2020).

Additivity of DIAAS

Additivity is the concept of combining different protein sources in a diet to improve the overall protein quality (Herreman et al., 2020). This is important because different protein sources have varying levels of indispensable AA and different digestibility of AA. Because individuals usually consume a meal with different combination of proteins, consumption of complementary proteins may increase protein quality when compared with consuming each protein source alone (Mariotti, 2017). Consuming a variety of protein sources can also provide other important nutrients such as vitamins and minerals, as well as help reduce the risk of nutrient deficiencies and certain chronic diseases (Mariotti and Gardner, 2019). When assessing protein quality using the DIAAS method, additivity in mixed meals should be evaluated because DIAAS allows for the calculation of the protein quality of a meal consisting of different proteins (FAO, 2014).

The DIAAS method is based on values for SID of AA that are measured in a protein source calculated by removing the variations of apparent ileal digestibility (**AID**) when using different ingredients in a diet (Stein et al., 2007). The SID provides a more accurate measurement of AA digestibility in foods and also allows for calculating additivity of values from individual ingredients when they are included in a mixed meal (Stein et al., 2005; Herreman et al., 2020). The basic assumption for additivity of values for SID is that the supply of digestible nutrients in a mixture of feed ingredients equals the sum of the supply determined from individual feed ingredients in a mixed diet (Stein et al., 2005). Therefore, it is also expected that DIAAS is additive in mixed meals, meaning that if values for individual ingredients are established, the DIAAS in mixed meals can be calculated. Values for SID are available for most feed ingredients used in animal diets, and some of these data may be transferred to a database for human foods to allow utilization of the DIAAS method.

While the concept of additivity, or combining different proteins in a meal, has been demonstrated for SID of AA in feed ingredients, this concept has not been demonstrated for human foods and DIAAS values of mixed meals have not been calculated. The optimal combination of proteins for different populations also needs to be identified. For example, there is a lack of information about the best protein combinations for the elderly, pregnant women, or athletes, and more research is needed to understand the specific needs of these populations (FAO, 2014). In addition, the food matrix and the impact of food processing and preparation methods on protein quality need to be evaluated because it can affect the digestibility of AA in foods and the additivity of AA in mixed meals (FAO, 2014). There is also a need to evaluate effects of additivity on dietary patterns and health outcomes such as weight management, muscle mass, and bone health (Mariotti and Gardner, 2019).

When developing dietary recommendations, it is important to consider cultural and traditional aspects regarding different protein sources that are available. Therefore, if a database on AA digestibility and DIAAS from different foods can be established, it will be possible to demonstrate the ability of a protein to complement another protein to produce a meal that is adequate in digestible AA. In addition, if DIAAS in different food combinations can be calculated from individual foods, it will allow a more practical approach for formulations of mixed meals that meet requirements of digestible AA (FAO, 2014).

Conclusion

Protein is an essential component of a healthy diet and plays an important role in many biological activities. The quality of a protein is determined by the concentration of indispensable AA and the ileal digestibility of each AA. Animal proteins typically have greater concentration of digestible AA than plant proteins. However, many people consume low-quality protein due to poverty or inadequate knowledge about nutrition. Specifically, many individuals suffer from protein-energy malnutrition, a severe global health issue. Therefore, ensuring adequate protein intake, particularly for vulnerable populations, is crucial for good health and prevention of chronic diseases. Strategies and policies are needed to ensure availability and affordability of high-quality protein for low-income populations.

Different methods for assessing protein quality in human foods have been developed, the most current being DIAAS. According to published data, values for DIAAS in animal-based proteins are often greater than in plant-based proteins, but alternative methods may be used to overcome this. Furthermore, the additivity approach in DIAAS ensures that the AA requirements are met. Although additional research is needed to fully understand the possible benefits and limitations of additivity, establishing a database for AA digestibility in different foods and calculating DIAAS in all foods and DIAAS in mixed meals, allow for a more practical approach to developing dietary recommendations.

Tables

Table 2.1. Digestible indispensable amino acid score (DIAAS) in animal-based proteins

Food item	DIAAS[1], %	Reference
Whey protein isolate	109 – 125	Mathai et al., 2017; Matsuoka et al., 2019
Whey protein concentrate	133	Mathai et al., 2017
Milk protein concentrate	141	Mathai et al., 2017
Casein	100 – 129	FAO 2013; Guillin et al., 2020
Porcine plasma hydrolysate	102	Bindari et al., 2017
Bovine muscle hydrolysate	81 (Trp)	Bindari et al., 2017
Ground beef	99 – 121 (Leu)	Bailey et al., 2020b
Ribeye roast	107 – 130	Bailey et al., 2020b
Salami	120	Bailey et al., 2020b
Beef topside steak	80 – 99 (Val)	Hodgkinson et al., 2018
Eggs	116 – 143	Matsuoka et al., 2019; Ertl et al., 2016
Bacon	117 – 142	Bailey et al., 2020c
Pork loin	117 – 139	Bailey et al., 2020c
Chicken meat	108	Ertl et al., 2016
Salmon fish powder	93 (Leu)	Desai et al., 2018
Insects	64 – 92 (SAA)[2]	Malla et al., 2022

[1] DIAAS range values are based on the reference pattern for individuals older than 3 years measured or calculated in foods from different studies. First-limiting amino acids (AA) in parentheses.

[2] SAA, sulfur amino acids.

Table 2.2. Digestible indispensable amino acid score (DIAAS) in plant-based proteins

Food item	DIAAS[1], %	Reference
Pea protein isolate	88 (SAA)[2]	Guillin et al., 2020
Pea protein concentrate	73 (SAA)	Mathai et al., 2017
Cooked peas	58 – 68 (SAA)	Rutherfurd et al., 2014; Ertl et al., 2016; Han et al., 2020
Soy protein isolate	90 – 98 (SAA)	Rutherfurd et al., 2014; Mathai et al., 2017
Soy flour	100 – 105	Ertl et al., 2016; Mathai et al., 2017
Wheat	20 – 68 (Lys)	Cervantes-Pahm et al., 2014; Ertl et al., 2016; Mathai et al., 2017
Cooked rice	60 (Lys)	Rutherfurd et al., 2014
Rice protein concentrate	37 (Lys)	Rutherfurd et al., 2014
Rice	42 – 64 (Lys)	Cervantes-Pahm et al., 2014; Han et al., 2019
Oats	43 – 77 (Lys)	Cervantes-Pahm et al., 2014; Abelilla et al., 2018; Han et al., 2019
Cooked beans	60 – 88 (Leu)	Han et al., 2020
Corn	42 – 54 (Lys)	Cervantes-Pahm et al., 2014; Ertl et al., 2016
Barley	47 – 51 (Lys)	Cervantes-Pahm et al., 2014; Ertl et al., 2016
Sorghum	29 (Lys)	Cervantes-Pahm et al., 2014
Pistachios	83 – 86 (Lys)	Bailey et al., 2020a

[1] DIAAS range values are based on the reference pattern for individuals older than 3 years measured or calculated in foods from different studies.

[2] SAA, sulfur amino acids.

Literature Cited

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

Aggarwal, A., and Drewnowski, A. 2019. Plant-and animal-protein diets in relation to sociodemographic drivers, quality, and cost: Findings from the Seattle Obesity Study. Am. J. Clin. Nutr. 110:451-460. doi:10.1093/ajcn/nqz064

Bailey, H. M., and Stein, and H. H. 2020a. Raw and roasted pistachio nuts (Pistacia vera L.) are ‘good’sources of protein based on their digestible indispensable amino acid score as determined in pigs. J. Sci. Food. Agric. 100:3878-3885. doi:10.1002/jsfa.10429

Bailey, H. M., Mathai, J. K., Berg, E. P., and Stein, H. H. 2020b. Most meat products have digestible indispensable amino acid scores that are greater than 100, but processing may increase or reduce protein quality. Br. J. Nutr. 24:14-22. doi:10.1017/S0007114520000641

Bailey, H. M., Mathai, J. K., Berg, E. P., and Stein, H. H. 2020c. Pork products have digestible indispensable amino acid scores (DIAAS) that are greater than 100 when determined in pigs, but processing does not always increase DIAAS. J. Nutr. 150:475-482. doi:10.1093/jn/nxz284

Batool, R., Butt, M. S., Sultan, M. T., Saeed, F., and Naz, R. 2015. Protein–energy malnutrition: A risk factor for various ailments. Crit. Rev. Food Sci. Nutr. 55:242-253. doi:10.1080/10408398.2011.651543

Bindari, Y. R., Lærke, H. N., and Nørgaard, J. V. 2017. 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

Bodwell, C. E., Carpenter, K. J., and McDonough, F. E. 1989. A collaborative study of methods of protein evaluation: introductory paper. Plant. Foods Hum. Nutr. 39:3-11. doi:10.1007/BF01092396

Cervantes-Pahm, S. K., Liu, Y., and Stein, H. H. 2014. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br. J. Nutr. 111:1663-1672. doi:10.1017/S0007114513004273

Desai, A. S., Brennan, M. A., and Brennan, C. S. 2018. Amino acid and fatty acid profile and digestible indispensable amino acid score of pasta fortified with salmon (Oncorhynchus tshawytscha) powder. Eur. Food Res. Technol. 244:1729-1739. doi:10.1007/s00217-018-3085-5

Domènech, L. 2015. Improving irrigation access to combat food insecurity and undernutrition: A review. Glob. Food Sec. 6:24-33. doi:10.1016/j.gfs.2015.09.001

Economic Research Service, US Department of Agriculture. 2019. Inflation-adjusted price indices for corn, wheat, and soybeans show long-term declines. https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=76964 (accessed Apr 2023)

Economic Research Service, US Department of Agriculture. 2023. Agricultural prices trended upward in 2021. https://www.ers.usda.gov/data-products/chart-gallery/gallery/chart-detail/?chartId=58360 (accessed Apr 2023)

Elango, R., Humayun, M. A., Ball, R. O., and Pencharz, P. B. 2010. Evidence that protein requirements have been significantly underestimated. Curr. Opin. Clin. Nutr. Metab. Care 13:52-57. doi:10.1097/MCO.0b013e328332f9b7

Ertl, P., Steinwidder, A., Schönauer, M., Krimberger, K., Knaus, W., and Zollitsch, W. 2016. Net food production of different livestock: a national analysis for Austria including relative occupation of different land categories. Environ. Plan. Manag. J. 67:91-103. doi:10.1515/boku-2016-0009

FAO/WHO. 1991. Protein quality evaluation. Report of the joint FAO/WHO expert consultation. FAO food and nutrition paper 51. Bethesda, MD.

FAO/WHO/ONU 2007. Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation. World Health Organization.

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. Rome.

FAO. 2014. Research approaches and methods for evaluating the protein quality of human foods. Report of an FAO expert working group. Rome.

FDA. 2018. 21CFR 101.9. Nutrition labeling of food. U.S Food and Drug Administration, Silver Springs, MD.

Finot, P. A. 1981. The extent of the Maillard reaction during the processing of milk. Prog. Food Nutr. Sci. 5:345-355.

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

Guillin, F. M., Gaudichon, C., Guérin-Deremaux, L., Lefranc-Millot, C., Azzout-Marniche, D., Khodorova, N., and Calvez, J. 2021. Multi-criteria assessment of pea protein quality in rats: A comparison between casein, gluten and pea protein alone or supplemented with methionine. Br. J. Nutr. 125:389-397. doi:10.1017/S0007114520002883

Han, F., Han, F., Wang, Y., Fan, L., Song, G., Chen, X., Jiang, P., Miao, H., and Han, Y. 2019. Digestible indispensable amino acid scores of nine cooked cereal grains. Br. J. Nutr. 121:30-41. doi:10.1017/S0007114518003033

Han, F., Moughan, P. J., Li, J., and Pang, S. 2020. Digestible indispensable amino acid scores (DIAAS) of six cooked Chinese pulses. Nutr. 12:3831. doi:10.3390/nu12123831

Herreman, L., Nommensen, P., Pennings, B., and Laus, M. C. 2020. Comprehensive overview of the quality of plant-And animal-sourced proteins based on the digestible indispensable amino acid score. Food Sci. Nutr. 8:5379-5391. doi:10.1002/fsn3.1809

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

Hodgkinson, S. M., Stein, H. H., de Vries, S., Hendriks, W. H., and Moughan, P. J. 2020. Determination of true ileal amino acid digestibility in the growing pig for calculation of digestible indispensable amino acid score (DIAAS). J. Nutr. 150:2621-2623. doi:10.1093/jn/nxaa210

Hoffman, J. R., and Falvo, M. J. 2004. Protein–which is best?. J. Sports Sci. Med. 3:118-130. PMID: 24482589.

Kong, C., and Adeola, O. 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian Australas. J. Anim. Sci. 27:917-925. doi:10.5713/ajas.2014.r.02

Lamb, M. W., and Harden, M. L. 1973. The meaning of human nutrition. Chapter 7: Protein as a source of amino acids. Editor: Maickel, R. 1st ed. Pergamon Press Inc. USA. p.153-191.

Lelijveld, N., Seal, A., Wells, J. C., Kirkby, J., Opondo, C., Chimwezi, E., Bunn, J., Bandsma, R., Heyderman, R. S., Nyirenda, M. J., and Kerac, M. 2016. Chronic disease outcomes after severe acute malnutrition in Malawian children (ChroSAM): a cohort study. Lancet Glob. Health. 4:e654-e662. doi:10.1016/S2214-109X(16)30133-4

Malla, N., Nørgaard, J. V., Lærke, H. N., Heckmann, L. H. L., and Roos, N. 2022. Some insect species are good-quality protein sources for children and adults: digestible indispensable amino acid score (DIAAS) determined in growing pigs. J. Nutr. 152:1042-1051. doi.org/10.1093/jn/nxac019

Mansilla, W. D., Marinangeli, C. P., Cargo-Froom, C., Franczyk, A., House, J. D., Elango, R., Columbus, D. A., Kiarie, E., Rogers, M., and Shoveller, A. K. 2020. Comparison of methodologies used to define the protein quality of human foods and support regulatory claims. Appl. Physiol. Nutr. Metab. 45:917-926. doi:10.1139/apnm-2019-0757

Mariotti, F., and Gardner, C. D. 2019. Dietary protein and amino acids in vegetarian diets—A review. Nutrients. 11:2661. doi:10.3390/nu11112661

Mariotti, F. 2017. Plant protein, animal protein, and protein quality. In: Vegetarian and plant-based diets in health and disease prevention. p.621-642. doi:10.1016/B978-0-12-803968-7.00035-6

Mathai, J. K., Liu, Y., and Stein, H. H. 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

Matsuoka, R., Kurihara, H., Nishijima, N., Oda, Y., and Handa, A. 2019. Egg white hydrolysate retains the nutritional value of proteins and is quickly absorbed in rats. Sci. World J. 2019. doi: 10.1155/2019/5475302

Mayen, A. L., Marques-Vidal, P., Paccaud, F., Bovet, P., and Stringhini, S. 2014. Socioeconomic determinants of dietary patterns in low-and middle-income countries: a systematic review. Am. J. Clin. Nutr. 100:1520-1531. doi:10.3945/ajcn.114.089029

Millward, D. J., Layman, D. K., Tomé, D., and Schaafsma, G. 2008. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. Am. J. Clin. Nutr. 87:1576S-1581S. doi:10.1093/ajcn/87.5.1576S

Mitchell, H. H. 1924. A method of determining the biological value of protein. J. Biol. Chem. 58:873-903. doi:10.1016/S0021-9258(18)85344-3

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

Osborne, T. B., Mendel, L. B., and Ferry, E. L. 1919. A method of expressing numerically the growth-promoting value of proteins. J. Biol. Chem. 37:223-229. doi:10.1016/S0021-9258(18)86353-0

Pellett, P. L., and Young, V. R. 1980. Nutritional evaluation of protein foods. Report of a working group sponsored by the International Union of Nutritional Sciences and the United Nations University World Hunger Programme (UNU/WHP). Food Nutr. Bull. (Suppl.) 4. ISBN:92-808-0129-5

Phillips, S. M. 2017. Current concepts and unresolved questions in dietary protein requirements and supplements in adults. Front. Nutr. 13. doi:10.3389/fnut.2017.00013

Rutherfurd, S. M., Fanning, A. C., Miller, B. J., and Moughan, P. J. 2014. 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 Ozimek, L. 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

Schlemmer, U., Frølich, W., Prieto, R. M., and Grases, F. 2009. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. Mol. Nutr. Food Res. 53:S330-S375. doi:10.1002/mnfr.200900099

Stein, H. H., Pedersen, C., Wirt, A. R., and Bohlke, R. A. 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., Seve, B., Fuller, M. F., Moughan, P. J., and de Lange, C. F. M. 2007. 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

Stein, H. H., Shipley, C. F., and Easter, R. A. 1998. Technical note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433-1436. doi:10.2527/1998.7651433x

UNICEF/WHO/World Bank Group. 2021. Level and trends in child malnutrition. Joint Child Malnutrition Estimates. https://data.unicef.org/resources/jme-report-2021/ (accessed Apr 2023)

Volpi, E., Kobayashi, H., Sheffield-Moore, M., Mittendorfer, B., and Wolfe, R. R. 2003. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am. J. Clin. Nutr. 78:250-258. doi:10.1093/ajcn/78.2.250

WFP, WHO, and UNICEF. 2022. The state of food security and nutrition in the world 2022. Report. https://data.unicef.org/resources/sofi-2022/ (accessed Apr 2023)

Witard, O. C., Garthe, I., and Phillips, S. M. 2019. Dietary protein for training adaptation and body composition manipulation in track and field athletes. Int. J. Sport Nutr. Exer. Metab. 29:165-174. doi:10.1123/ijsnem.2018-0267

Wu, G. 2016. Dietary protein intake and human health. Food Funct. 7:1251-1265. doi:10.1039/c5fo01530h

CHAPTER 3: VALUES FOR DIGESTIBLE INDISPENSABLE AMINO ACID SCORE (DIAAS) DETERMINED IN PIGS ARE GREATER FOR MILK THAN FOR BREAKFAST CEREALS, BUT DIAAS VALUES FOR INDIVIDUAL INGREDIENTS ARE ADDITIVE IN COMBINED MEALS[1]

Abstract

Breakfast cereals contain low-quality proteins and are often consumed with milk. The digestible indispensable amino acid score (**DIAAS**) has been used to evaluate protein quality, but it is not known if DIAAS obtained in individual foods is additive in combined meals. The following hypotheses were tested: amino acids (**AA**s) in milk complement AAs in breakfast cereals to provide a balanced meal, and DIAAS in individual foods is additive in combined meals. Six ileal cannulated gilts [body weight mean: 55.6 ± 3.7 (SD) kg] were allotted to a 6 × 6 Latin square with six 7-d periods. Ileal digesta were collected for 9 h on days 6 and 7 of each period. Three diets contained a breakfast cereal (i.e., cornflakes or quick oats) or dry milk as the sole source of AAs. Two additional diets contained a combination of dry milk and cornflakes or quick oats. A nitrogen-free diet was also used, and DIAAS was calculated for cornflakes, quick oats, dry milk, and the 2 combined meals for children aged 6 to 36 mo and individuals older than 36 mo through adulthood. For the combined meals, DIAAS was also predicted from the individual ingredient DIAAS. Dry milk had greater ($P < 0.05$) DIAAS (123 and 144) than quick oats (57 and 67), but cornflakes had less ($P < 0.05$) DIAAS (16 and 19) than the other ingredients. Both breakfast

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[1] Material from: Natalia S. Fanelli et al., Values for digestible indispensable amino acid score (DIAAS) determined in pigs are greater for milk than for breakfast cereals, but DIAAS values for individual ingredients are additive in combined meals, The Journal of Nutrition, published 2021, publisher: Elsevier - Oxford University Press on behalf of the American Society for Nutrition. The copyright owner has provided permission to reprint.

cereal–dry milk meals had DIAAS close to or greater than 100 for children aged 6 mo to 3 y and for older children, adolescents, and adults, but there were no differences between measured and predicted DIAAS. The combination of milk and breakfast cereals results in a meal that is balanced in indispensable AAs for humans, and DIAAS obtained from individual ingredients is additive in mixed meals.

Key words: amino acids, breakfast cereals, digestible indispensable amino acid score, milk, protein digestibility

Abbreviations: AA, amino acids; AACF, amino acids in cornflakes; AAM, amino acids in milk; AID, apparent ileal digestibility; AIDCF, apparent ileal digestibility in cornflakes; AIDM, apparent ileal digestibility in milk; AIDP, predicted apparent ileal digestibility; CP, crude protein; DIAAS, digestible indispensable amino acid score; FAO, Food and Agriculture Organization; SID, standardized ileal digestibility.

Introduction

Almost 50% of American children consume cereals for breakfast (Schwartz et al., 2008). Diets based only on cereals have low protein quality (Shewry, 2007), and to meet requirements for amino acids (**AA**s), higher-quality proteins are needed to complement the protein in cereals to provide a meal that is adequate in all indispensable AAs (Mathai et al., 2017). Breakfast cereals are often consumed in combination with milk, and it is assumed that the combined cereal-milk meal meets requirements for all AAs, although to our knowledge, data to demonstrate this have not been published.

The digestible indispensable amino acid score (**DIAAS**) may be used to determine protein quality (FAO, 2013). The method is based on AA digestibility determined for each AA at the distal ileum, and the pig has been accepted as an appropriate animal model for estimating AA digestibility if values cannot be determined in humans (FAO, 2013, Shivakumar et al., 2019). The DIAAS method allows for calculation of the protein value of individual ingredients and mixed meals consisting of several proteins (FAO, 2013).

Values for DIAAS are based on values for apparent ileal digestibility (**AID**) of AAs that are corrected for the basal endogenous loss of each individual AA, resulting in values defined as standardized ileal digestibility (**SID**). Values for SID of AAs are additive in mixed diets because these values are independent of basal endogenous losses (Stein et al., 2005, Xue et al., 2014). As a consequence, it is expected that DIAAS obtained for individual food ingredients is additive in a mixed meal, but data to demonstrate this have not been reported. Therefore, the objective of this experiment was to test the hypothesis that milk can be used to complement breakfast cereals to produce a meal that meets requirements for AAs. The second hypothesis was that DIAAS, in combined meals consisting of a breakfast cereal and milk, can be calculated from DIAAS values obtained in individual ingredients.

Materials and Methods

The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois before the experiment was conducted. Pigs that were the offspring of L359 males mated to Camborough females (PIC) were used in the experiment.

Animals and experimental design

Six growing gilts [initial body weight mean: 55.6 ± 3.7 (SD) kg] were surgically equipped with a T-cannula in the distal ileum using procedures adapted from Stein et al. (Stein et al., 1998). Pigs were allotted to a 6 × 6 Latin square design with 6 diets and six 7-d periods. Pigs were housed individually in pens (1.5 × 2.5 m) in an environmentally controlled room. Each pen had smooth sides and partially slatted floors. A nipple drinker and a feeder were also installed in each pen. At the conclusion of the experiment, pigs had a body weight mean of 85.1 ± 7.7 (SD) kg.

Diets, feeding, and sample collection

Two breakfast cereals (i.e., quick oats and cornflakes) and a lyophilized nonfat dry milk powder were procured (Table 3.1). The quick oats and the nonfat dry milk were purchased from Augason Farms Company, and the cornflakes were from Kellogg's Company. Three diets were based on quick oats, cornflakes, or dry milk as the only source of crude protein (**CP**) and AAs. Two additional diets were based on a combination of milk and quick oats or milk and cornflakes. A nitrogen-free diet was used to determine basal endogenous losses of CP and AAs to enable the calculation of SID of CP and AAs and calculation of DIAAS (Tables 3.2 and 3.3). Vitamins and minerals were included in the diets to meet or exceed current nutrient requirement estimates for growing pigs (NRC, 2012).

The dry milk diet and the nitrogen-free diet were mixed as complete diets with all ingredients included. However, 4 different premixes containing minerals, titanium dioxide, cornstarch, sugar, and salt were prepared and added to the cornflakes diet, the quick oats diet, and the cornflakes or quick oats–dry milk mixtures prior to feeding. All diets, except the nitrogen-free diet and the cornflakes diet, were fed in a liquid form. The diet containing cornflakes as the only source of AAs was prepared by grinding the cornflakes in a food processor (4-Quart Food Processor with LiquiLock Seal System, WFP16SCND; Waring Commercial) and mixing the ground cornflakes with its designated premix. The cornflakes diet contained more sugar because it was necessary to improve palatability of this diet. The cornflakes–dry milk diet was prepared by manually grinding the cornflakes, adding the dry milk (proportion of 2.6:1 cornflakes–dry milk) and the designed premix, and then mixing with water at a ratio of 1:4. The diet containing the dry milk as the only source of AAs was prepared by mixing the whole diet portion with water at a ratio of 1:3.

The quick oats diet was prepared as a porridge by mixing with water at a ratio of 1:3, and this mixture was then heated on a hotplate until boiling, after which time it was removed from the hotplate and left at room temperature to cool. When the porridge had a temperature of ~20°C, it was mixed with the designated premix and offered to the pigs. The quick oats–dry milk diet was prepared the same way, but the dry milk was added when mixing the porridge with the premix (proportion of 2.4:1 quick oats–dry milk). The dry milk was added in different proportions in the mixed diets due to difficulty of mixing the cornflakes with dry milk.

A sample of cornflakes, quick oats, and dry milk and of all diets was collected at the time of diet mixing, and this sample was used for chemical analysis. All diets were fed to 1 pig in each period, and no pig received the same diet more than once during the experiment. There were, therefore, 6 replicate pigs per treatment. Pig weights were recorded at the beginning of each period to calculate feed allowance during the following period, and the amount of feed supplied each day was recorded. All pigs were fed their assigned diets in a daily amount equivalent to 4% of body weight in 2 equal meals that were provided every day at 08:00 and 17:00, and water was available at all times. Feed refusals were recorded daily and zootechnical performance was calculated (Table 3.4). Pig weights were also recorded at the conclusion of the experiment. The initial 5 d of each period were considered the adaptation period to the diet, and ileal digesta were collected from 08:00 to 17:00 on days 6 and 7 using standard procedures (Stein et al., 1998). On the completion of 1 experimental period, animals were deprived of feed overnight, and the following morning, a new experimental diet was offered.

Chemical analysis

At the conclusion of the experiment, ileal digesta samples were thawed and mixed within animal and diet, and a subsample was lyophilized and finely ground prior to analysis. Subsamples of diets were also lyophilized and finely ground before analysis. Samples of all ingredients, diets, and ileal digesta were analyzed for nitrogen by combustion [Method 990.03 (AOAC, 2007)] using a LECO FP628 analyzer (LECO Corp.) and for AAs [Method 982.30 E (a, b, c) (AOAC, 2007)]. The CP was calculated as nitrogen × 6.25. Dry matter was analyzed in the dry milk and in the diets containing milk as described by Ahn et al. (2014), but Method 930.15 (AOAC, 2007) was used to analyze dry matter in diets, ingredients, and ileal digesta samples that did not contain milk. Diets and ingredients were analyzed for ash [Method 942.05 (AOAC, 2007)], and gross energy was analyzed using an isoperibol bomb calorimeter (Model 6300; Parr Instruments) with benzoic acid as the standard for calibration. Acid-hydrolyzed ether extract was analyzed by acid hydrolysis using 3N HCl (Ankom^{HCl}; Ankom Technology) followed by crude fat extraction using petroleum ether (Ankom^{XT15}; Ankom Technology). Cornflakes and quick oats were also analyzed for insoluble dietary fiber and soluble dietary fiber [Method 991.43 (AOAC, 2007)] using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology). Starch was analyzed in cornflakes and quick oats using the glucoamylase procedure [Method 979.10 (AOAC, 2007)]. Diets and ileal digesta samples were also analyzed for titanium (Myers et al., 2004).

Calculations

The AID and SID of CP and all AAs in each diet were calculated using published equations (Stein et al., 2007). The predicted AID of AAs in the diet containing cornflakes and dry milk was calculated using the following equation (Stein et al., 2005):

$$\text{AIDP} = [(\text{AACF} \times \text{AIDCF}) + (\text{AAM} \times \text{AIDM})] / (\text{AACF} + \text{AAM})$$

where AIDP (%) is the predicted AID for an AA in the mixed diet, and AACF and AAM are the concentrations (%) of that AA contributed by cornflakes and dry milk, respectively, which were calculated by multiplying the concentration of that AA (%) in the ingredient by the proportion (%) of the ingredient in the mixed diet. The determined AID (%) of the AA in cornflakes and dry milk was designated as AIDCF and AIDM, respectively. The predicted AID of CP and the SID of CP and all AAs in the diet containing cornflakes and dry milk were calculated using the same equation. Likewise, values for the predicted AID and SID of CP and all AAs in the quick oats–dry milk diet were also calculated using this equation. In the calculations of predicted values for AID and SID in the mixed diets, no effects of period were observed; therefore, values obtained from the same pig for each ingredient were used.

The DIAAS reference ratio for each protein source or mixed diet was calculated using the following equation (Cervantes-Pahm et al., 2014):

Digestible indispensable AA reference ratio = mg digestible indispensable AA content in 1 g protein of food/mg of the same dietary indispensable AA in 1 g of the reference protein.

Separate reference ratios were calculated for 2 age groups: children from 6 to 36 mo and for older children, adolescents, and adults (FAO, 2013).

The DIAAS values were also calculated for these age groups as recommended by Food and Agriculture Organization (**FAO**) (2013) using the following equation:

DIAAS (%) = 100 × lowest value of the digestible indispensable AA reference ratio

Statistical analysis

The number of replicates per treatment was based on recommendations from FAO (2014). At the conclusion of the experiment, normality of residuals was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Institute). Data were analyzed by ANOVA using the PROC MIXED procedure in SAS. The pig was the experimental unit for all analyses. Diet was the fixed effect, and pig and period were random effects. Treatment means were calculated using the LSMEANS statement in SAS, and when significant, means were separated using the PDIFF option in the MIXED procedure. A t test was used to test the null hypothesis that the difference between the determined and predicted AID or SID of CP and AAs, as well as DIAAS for the mixed diets, was equal to 0. Significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Health of pigs was evaluated daily by the investigators and the farm staff and followed a protocol approved by the Animal Care and Use Committee at the University of Illinois. All pigs remained healthy during the experimental period, and only little feed refusals were observed.

The AID for CP and all AAs was greater ($P < 0.05$) in dry milk than in cornflakes (Table 3.5). Likewise, the AID for CP and all AAs was greater ($P < 0.05$) in dry milk than in quick oats, except for Arg, Cys, and Ser. Cornflakes also had less ($P < 0.05$) AID for CP and all AAs compared with quick oats, except for Ala. The SID for CP, Ile, Met, Thr, Val, Ala, and Glu was greater ($P < 0.05$) in cornflakes and dry milk than in quick oats, and dry milk had greater ($P <$

0.05) SID for His, Leu, Lys, Phe, Asp, Cys, and Tyr than both cereals. Quick oats had lower (*P* < 0.05) SID of Trp than dry milk and also had lower (*P* < 0.05) SID of Ser than cornflakes.

Differences between measured and predicted AID in the cornflakes– dry milk diet differed (*P* < 0.05) from zero for CP, Arg, Met, Ala, Asp, and Tyr and tended (0.05 ≤ *P* < 0.10) to differ from zero for His, Lys, Phe, and Thr (Table 3.6). Differences between measured and predicted values for SID differed (P < 0.05) from zero for Ile, Glu, and Ser and tended (0.05 ≤ *P* < 0.10) to differ from zero for Leu, Val, and Cys. However, for all other AAs, no differences between measured and predicted values for AID and SID of AA in the cornflakes–dry milk diet were observed. For the quick oats–dry milk diet, the AID and SID of Ser tended (0.05 ≤ *P* < 0.10) to be different between measured and predicted values (Table 3.7), but for CP and all other AAs, no differences between measured and predicted values for AID and SID were observed in the quick oats–dry milk diet.

For children aged 6 mo to 3 y and for older children, adolescents, and adults, dry milk had greater (*P* < 0.05) DIAAS than the other diets, whereas cornflakes had lower (*P* < 0.05) DIAAS than the other diets (Table 3.8). The first limiting AA for both age groups was Lys in cornflakes and quick oats, but there was no limiting AA in dry milk because DIAAS values >100 are not considered limiting. For both age groups, there was no limiting AA for the cornflakes-dry milk combination. Likewise, there was no limiting AA for the quick oats-dry milk combination for individuals older than 36 mo, however, for children from 6 to 36 mo, Lys was the first limiting AA for this combined meal (Table 3.9). The measured and predicted reference values for cornflakes–dry milk differed (*P* < 0.05) from zero for Ile and tended (0.05 ≤ *P* < 0.10) to differ from zero for Leu, sulfur amino acid, and Val. In contrast, no differences between the predicted and the measured reference values for any AA in the quick oats–dry milk combination were observed. Regardless of age group, there were no differences between predicted and measured DIAAS values.

Discussion

The nutrient composition of cornflakes, quick oats, and dry milk was generally within the range of published values (USDA Database, 2020) for these food ingredients. Cornflakes had lower CP, AA, total dietary fiber, and acid- hydrolyzed ether extract compared with corn (NRC, 2012) and was also very low in Lys, indicating that the cornflakes may have been heat damaged because overheating reduces the concentration of Lys in foods (Finot, 1981). The observation that the AID for Lys in cornflakes was negative and the SID of Lys was the lowest among all AAs also indicates that the cornflakes used in this experiment were heat damaged. However, SID of CP and most AAs, except Lys, in cornflakes were greater than reported for corn (NRC, 2012; Cervantes-Pahm, 2014), which is likely due to processing and to the lower concentration of total dietary fiber in cornflakes compared with corn. It is also possible that the cornflakes being ground prior to feeding may have affected the SID of AAs, but it was necessary to grind cornflakes to make the pigs consume them. The SID of CP and AAs that were measured for quick oats and dry milk were within the range of reported values for dehulled oats and dry skim milk, respectively (NRC, 2012; Cervantes-Pahm, 2014; Mathai et al., 2017).

The observation that only few differences between the measured and the predicted SID of AAs were observed for the cornflakes–dry milk diet, and none for the quick oats–dry milk diet, demonstrates that SID values in mixed meals can be predicted from individual ingredients as previously demonstrated for other types of diets (Stein et al., 2005; Xue et al., 2014). The reason there were a few differences between measured and predicted values for AID of AAs in the cornflakes–dry milk diet is that values for AID are not always additive in mixed diets if the concentration of AAs in one of the ingredients is less than in the mixed diet (Stein et al., 2005). The reason no differences between the measured and predicted AID in the quick oats–dry milk diet were observed likely is that the concentration of CP and AAs in the quick oats diet, the dry milk diet, and the quick oats–dry milk diet was not different, and if that is the case, AID values are also expected to be additive in mixed diets (Stein et al., 2005).

The DIAAS for cornflakes was very low for both age groups, and Lys was the first limiting AA, which is due to the low concentration and the low SID of Lys that was determined for this ingredient. The reason Trp was the second limiting AA in cornflakes is that corn protein is always low in Lys and Trp (Rafii et al., 2018). Lys is also the first limiting AA in whole corn (Cervantes-Pahm, 2014), but the reported DIAAS in whole corn is greater than the DIAAS for cornflakes determined in this experiment, which is likely because of the heat damage that reduced the DIAAS of cornflakes. A very low DIAAS for a corn-based breakfast cereal was also reported previously further, indicating that the processing used to prepare breakfast cereals may result in reduced digestibility of Lys (Rutherfurd et al., 2014). The observation that Lys was also the first limiting AA in quick oats is in agreement with data for dehulled oats and oat protein concentrate, and the DIAAS in quick oats is close to the DIAAS for dehulled oats and oat protein concentrate (Cervantes-Pahm, 2014; Abelilla et al., 2018; Han et al., 2019). The high DIAAS in dry milk is consistent with data reported by Mathai et al. (2017) and by Rutherfurd et al. (2014), and this reflects the greater protein quality in milk compared with cereal grains and breakfast cereals produced from cereal grains.

Values for DIAAS in grain-based diets are defined by the Lys concentration and digestibility because Lys is first limiting in all grain products (Cervantes-Pahm, 2014). This represents a problem in foods that have undergone early or advanced Maillard reactions during processing. Advanced Maillard reaction products will not be analyzed as Lys, but early Maillard reaction products may revert back to Lys during acid hydrolysis prior to AA analysis (Kim et al., 2012; FAO, 2013). The AA analysis, therefore, may overestimate Lys concentration, and therefore also DIAAS, in these ingredients. The observation that the AID of Arg in cornflakes was very low indicates that Arg, which also contains amino groups in the side chain, may also have been heat damaged during processing. It is, however, also possible that the diet-specific endogenous loss of Arg was greater when pigs were fed the cornflakes diet, which also may have contributed to the low AID of Arg.

The DIAAS of almost 100 for children aged 6 to 36 mo and >100 for individuals older than 36 mo indicates that the combination of cornflakes or quick oats and milk provides a balanced ratio of AAs, and if consumed in sufficient quantities, this combination will meet dietary requirements for AAs for those age groups. The reason DIAAS in the cornflakes–dry milk meal was greater than in the quick oats–dry milk meal, despite the greater DIAAS for quick oats compared with cornflakes, is that the inclusion of milk was greater in the cornflakes–dry milk diet than in the quick oats–dry milk diet.

Results of this experiment confirm that the high protein quality in milk can compensate for low protein quality in cereal grains if milk and cereal grains are combined. Because Lys was the AA in least concentration in both meals, the daily intake of the meal to meet AA requirements is defined by the intake of Lys. If it is assumed that human adults have a daily Lys requirement of 30 mg/kg body weight (WHO, 2007) and that one-third of the daily Lys requirement needs to be satisfied by eating breakfast, an 80-kg adult human will need to consume ~115 g of the cornflakes-milk meal or 100 g of the quick oats–milk meal to meet the requirement for all AAs.

One advantage of using DIAAS to evaluate protein quality is that values are not truncated to 100, which allows for calculation of complementary effects of high-quality proteins in mixed meals, as demonstrated in this experiment. Because most individuals eat meals that consist of several proteins, it is important that values for protein quality of individual ingredients are additive in mixed meals because that allows for calculation of the protein quality of mixed meals from the quality of individual ingredients. Although fat-free milk powder was used in this experiment, it is likely that results can be extrapolated to diets containing breakfast cereals and full-fat or partially defatted milk products. If anything, fat-containing milk may have slightly greater DIAAS than the nonfat milk used in this experiment because dietary fat increases AA digestibility due to slower gastric emptying (Cervantes-Pahm and Stein, 2008).

The demonstrated additivity of SID values for most indispensable AAs is in agreement with previous data (Stein et al., 2005; Xue et al., 2014). The observation that among all the indispensable AAs, only the branched-chain AAs in the cornflakes-milk diet tended to not be different may be a result of the Leu-induced catabolism of Ile and Val that may take place if Leu is present in concentrations greater than the requirement, as is often the case in diets based on corn protein (Kwon et al., 2019).

The present data demonstrate, to our knowledge, for the first time that DIAAS values for individual ingredients are additive in mixed meals. The implication of this observation is that if a database for DIAAS in individual food ingredients can be established, it is possible to predict DIAAS in combinations of different foods. Therefore, it will be beneficial if DIAAS can be determined in more food proteins. Although the principles for additivity of DIAAS in mixed diets demonstrated in this research is believed to be applicable to all types of mixed meals, additional research to demonstrate additivity of DIAAS in other combinations of meals should be conducted.

In some cases, it may not be possible to use DIAAS values for foods that are processed exactly as the foods used in a specific situation, and the question, then, is if DIAAS obtained in foods processed in a different way can be used to calculate DIAAS in a combined meal. To address this question, DIAAS values for the combined meals of cornflakes and milk or quick oats and milk were calculated using published DIAAS values for a corn-based breakfast cereal and cooked rolled oats as well as for skimmed milk powder (Rutherfurd et al., 2014; Mathai et al., 2017). Results of these calculations indicated that the cornflakes-milk DIAAS based on published values was less than calculated in this experiment (91 and 108 for children aged 6–36 mo and individuals aged >36 mo, respectively, compared with the DIAAS of 101 and 120 obtained in this experiment). For the quick oats–milk diet, the calculated values were 97 and 115 for children aged between 6 and 36 mo and individuals aged >36 mo, respectively, and these values were in very good agreement with the values calculated in this experiment (95 and 113, respectively). It thus appears that under certain circumstances, values for DIAAS in a mixed meal may be calculated using values for slightly different ingredients.

Whereas calculations of DIAAS values for food proteins represent a significant improvement in evaluation of food proteins compared with previously used methods (FAO, 2013; Mathai et al., 2017), diets for monogastric livestock and companion animals are usually not formulated based on protein scores. Instead, mixed diets for animals are formulated by matching SID values for each indispensable AA obtained in each ingredient and calculated for the mixed diet, to the requirement of each individual AA. This is a more logical and accurate procedure than assigning a protein score to each ingredient. However, because SID values for food proteins are generated in the calculation of DIAAS, it will be possible to use these values if, at a later time, human food protein evaluation will be based directly on SID values for each AA.

Conclusion

Data from this experiment demonstrate that regardless of age group, cornflakes or quick oats are low-quality proteins, and individuals consuming only these grains will receive an unbalanced supply of indispensable AAs. However, when younger children, older children, adolescents, and adults consume a combination of breakfast cereals and milk, they will consume a meal with a DIAAS close to or >100, indicating that the high protein quality of milk complements the low protein quality of cereals to generate a diet that is balanced in indispensable AAs. Results also demonstrated that DIAAS obtained for individual ingredients is additive in mixed meals when fed to growing pigs, and the implication of this observation is that DIAAS in combined meals can be calculated from values obtained in individual ingredients.

Tables

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

Item, %	Corn flakes	Quick oats	Dry milk
Dry matter	96.00	89.01	95.76
Gross energy (kcal/kg)	3,975	4,235	4,084
CP	5.85	16.63	34.30
AEE	1.07	7.10	0.60
Ash	1.84	1.50	7.63
Carbohydrates			
Lactose[2]	-	-	53.23
Starch	74.61	50.68	-
IDF	2.80	5.50	-
SDF	-	4.30	-
TDF	2.80	9.80	-
Indispensable AA			
Arg	0.15	1.06	1.18
His	0.17	0.36	0.97
Ile	0.24	0.63	1.86
Leu	0.92	1.19	3.38
Lys	0.07	0.65	2.81
Met	0.11	0.26	0.86
Phe	0.34	0.86	1.71
Thr	0.19	0.51	1.44

Table 3.1 (cont.)

Trp	0.03	0.18	0.50
Val	0.28	0.83	2.20
Total	2.50	6.53	16.91
Dispensable AA			
Ala	0.49	0.72	1.11
Asp	0.35	1.25	2.64
Cys	0.11	0.50	0.29
Glu	1.31	3.30	7.40
Gly	0.18	0.75	0.64
Pro	0.64	0.81	3.23
Ser	0.28	0.66	1.59
Tyr	0.26	0.56	1.66
Total	3.62	8.55	18.56
Total AA	6.12	15.08	35.47

[1] AA, amino acids; AEE, acid hydrolyzed ether extract; CP, crude protein; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber.

[2] Lactose was calculated by difference of dry matter, CP, AEE, and ash.

Table 3.2. Ingredient composition of the 6 experimental diets (as-fed basis) fed to growing pigs[1]

Item, %	Corn flakes	Quick oats	Dry milk	Corn flakes-dry milk	Quick oats-dry milk	Nitrogen-free
Corn flakes	57.4	-	-	65.0	-	-
Quick oats	-	75.0	-	-	40.0	-
Dry milk	-	-	36.0	25.0	17.0	-
Corn starch	-	12.5	46.6	3.35	30.85	68.25
Solka floc	-	-	4.00	-	-	4.00
Soy oil	-	-	2.00	-	-	4.00
Limestone	0.85	1.35	0.45	0.40	0.70	0.50
Dicalcium phosphate	1.20	0.20	-	0.70	0.50	1.80
Sodium chloride	-	0.40	0.40	-	0.40	0.40
Magnesium oxide	-	-	-	-	-	0.10
Potassium carbonate	-	-	-	-	-	0.40
Sucrose	40.0	10.0	10.0	5.00	10.0	20.0
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40

Table 3.2 (cont.)

Vitamin mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15

[1] The dry-milk and N-free diets were mixed as complete diets, whereas premixes consisting of starch, sugar, minerals, vitamins, and TiO2 were prepared separately for the cereal diets and the cereal-dry milk combination diets.

[2] 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 d,l- α-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 the 6 experimental diets (as-fed basis) fed to growing pigs[1]

Item, %	Corn flakes	Quick oats	Dry milk	Corn flakes-dry milk	Quick oats-dry milk	Nitrogen-free
Dry matter	96.55	98.59	93.33	95.67	97.41	93.04
Gross energy (kcal/kg)	3,780	4,400	4,005	3,908	4,246	3,989
CP	3.75	13.32	12.81	12.07	13.80	0.33
AEE	0.56	5.87	1.75	0.29	3.49	2.75
Ash	4.39	2.88	3.51	4.69	3.01	2.73
Indispensable AA						
Arg	0.07	0.89	0.46	0.35	0.71	0.01
His	0.09	0.30	0.37	0.32	0.34	0.00
Ile	0.13	0.52	0.71	0.57	0.63	0.01
Leu	0.52	1.01	1.33	1.33	1.18	0.03
Lys	0.04	0.55	1.10	0.70	0.82	0.02
Met	0.05	0.22	0.35	0.27	0.29	0.01
Phe	0.19	0.73	0.67	0.59	0.72	0.01

Table 3.3 (cont.)

Thr	0.11	0.44	0.58	0.46	0.51	0.01
Trp	0.03	0.12	0.18	0.13	0.15	0.02
Val	0.16	0.69	0.86	0.68	0.79	0.01
Total	1.39	5.47	6.61	5.40	6.14	0.13
Dispensable AA						
Ala	0.28	0.61	0.45	0.54	0.55	0.01
Asp	0.19	1.06	1.05	0.82	1.08	0.02
Cys	0.07	0.41	0.11	0.14	0.29	0.00
Glu	0.74	2.88	2.97	2.52	2.97	0.03
Gly	0.10	0.64	0.26	0.26	0.49	0.01
Pro	0.34	0.71	1.31	1.14	0.98	0.02
Ser	0.16	0.59	0.66	0.55	0.63	0.01
Tyr	0.10	0.43	0.55	0.46	0.48	0.01
Total	1.98	7.33	7.36	6.43	7.47	0.11
Total amino acids	3.37	12.80	13.97	11.83	13.61	0.24

[1] AA, amino acids; AEE, acid hydrolyzed ether extract; CP, crude protein.

Table 3.4. Growth performance of the 6 experimental diets fed to growing pigs[1]

Item, %	Corn flakes	Quick oats	Dry milk	Corn flakes-dry milk	Quick oats-dry milk	N-free	SEM	*P*-value
n[2]	5	6	6	6	6	6		
Initial weight (kg)	69.56	65.48	66.55	63.95	64.77	68.33	4.38	0.310
Final weight (kg)	66.88^{b}	74.62^{a}	72.08^{a}	74.37^{a}	73.32^{a}	65.33^{b}	4.71	<0.001
ADG (kg/d)	-0.36^{c}	1.31^{a}	0.79^{b}	1.49^{a}	1.22^{a}	-0.43^{c}	0.13	<0.001
ADFI (kg/d)	1.76^{c}	2.44^{a}	2.27^{ab}	2.38^{a}	2.39^{a}	2.04^{b}	0.14	0.0002
G:F	-0.20^{c}	0.53^{a}	0.35^{b}	0.62^{a}	0.51^{a}	-0.22^{c}	0.05	<0.001

[1] Values are means and pooled SEMs. ADFI, average daily feed intake; ADG, average daily gain; G:F, gain to feed conversation ratio.

[2] *n* indicates the number of replicates for each item within each treatment.

Table 3.5. Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) for ingredients fed to growing pigs[1]

	AID					SID				
Item, %	Corn flakes	Quick oats	Dry milk	SEM	*P*-value	Corn flakes	Quick oats	Dry milk	SEM	*P*-value
n[2]	6	6	6			6	6	6		
CP	55.3^{c}	79.4^{b}	89.5^{a}	1.39	<0.001	98.6^{a}	91.9^{b}	101.8^{a}	1.39	0.007
Indispensable AA										
Arg	4.6^{b}	86.1^{a}	93.1^{a}	5.56	<0.001	109.1	94.5	108.4	5.56	0.160
His	68.6^{c}	81.7^{b}	94.8^{a}	1.15	<0.001	87.7^{b}	87.5^{b}	99.3^{a}	1.15	<0.001
Ile	68.7^{c}	80.7^{b}	91.2^{a}	1.25	<0.001	92.5^{a}	86.7^{b}	95.4^{a}	1.25	0.0003
Leu	87.8^{b}	83.3^{c}	95.2^{a}	0.63	<0.001	96.9^{b}	88.1^{c}	98.6^{a}	0.63	<0.001
Lys	-13.6^{c}	75.9^{b}	93.1^{a}	4.23	<0.001	77.9^{b}	82.7^{b}	96.3^{a}	4.23	0.018
Met	81.0^{c}	84.9^{b}	96.2^{a}	0.98	<0.001	97.7^{a}	88.8^{b}	98.5^{a}	0.98	<0.001
Phe	80.1^{c}	84.4^{b}	95.2^{a}	0.78	<0.001	94.8^{b}	88.4^{c}	99.3^{a}	0.78	<0.001
Thr	49.9^{c}	73.7^{b}	87.7^{a}	2.17	<0.001	93.1^{a}	84.7^{b}	95.6^{a}	2.17	0.003
Trp	66.3^{c}	78.8^{b}	92.8^{a}	2.63	0.0004	91.4^{ab}	85.2^{b}	96.8^{a}	2.63	0.024

Table 3.5 (cont.)

Val	63.2^{c}	78.5^{b}	91.0^{a}	1.38	<0.001	94.3^{a}	85.9^{b}	96.6^{a}	1.38	<0.001
Dispensable AA										
Ala	76.4^{b}	77.1^{b}	87.3^{a}	1.70	0.029	97.6^{a}	87.0^{b}	100.0^{a}	1.70	0.023
Asp	48.9^{c}	79.4^{b}	90.9^{a}	1.62	<0.001	87.2^{b}	86.4^{b}	97.6^{a}	1.62	0.0002
Cys	62.4^{b}	84.3^{a}	80.5^{a}	2.14	<0.001	92.6^{b}	89.6^{b}	99.1^{a}	2.14	0.013
Glu	84.3^{c}	89.3^{b}	93.5^{a}	0.68	<0.001	95.8^{a}	92.3^{b}	96.3^{a}	0.68	0.002
Ser	69.5^{b}	82.9^{a}	85.9^{a}	1.13	<0.001	95.3^{a}	90.1^{b}	91.9^{ab}	1.13	0.019
Tyr	71.3^{c}	82.4^{b}	94.7^{a}	0.99	<0.001	95.4^{b}	88.1^{c}	99.0^{a}	0.99	<0.001

[1] Values are means and pooled SEMs. Labeled means in a row without a common superscript letter differ, $P < 0.05$. AID, apparent ileal disgestibility; SID, standardised ileal digestibility. SID 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 N-free diet as follows (g/kg DM intake): CP, 16.83; Arg, 0.76; His, 0.18; Ile, 0.32; Leu, 0.49; Lys, 0.38; Met, 0.09; Phe, 0.29; Thr, 0.49; Trp, 0.08; Val, 0.52; Ala, 0.61; Asp, 0.75; Cys, 0.22; Glu, 0.89; Ser, 0.43; Tyr, 0.25.

[2] n indicates the number of replicates for each item within each treatment.

Table 3.6. Measured and predicted values for apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in the corn flakes-dry milk meal-based diet fed to growing pigs[1]

	AID				SID			
Item, %	Measured	Predicted	Difference	SEM	Measured	Predicted	Difference	SEM
n[2]	6	6			6	6		
CP	85.1	79.0	6.1**	1.31	98.4	100.8	-2.4	1.31
Indispensable AA								
Arg	88.0	71.1	16.9**	2.92	108.7	108.6	0.1	2.92
His	88.8	86.6	2.2^{+}	1.06	94.1	95.7	-1.6	1.06
Ile	86.0	85.5	0.5	0.91	91.4	94.6	-3.2*	0.91
Leu	93.0	92.1	1.0	0.61	96.6	97.9	-1.3^{+}	0.61
Lys	89.1	86.6	2.5^{+}	1.19	94.3	95.2	-0.9	1.19
Met	95.0	92.4	2.6**	0.53	98.0	98.3	-0.3	0.53
Phe	91.9	90.1	1.8^{+}	0.74	96.6	97.8	-1.2	0.74
Thr	82.1	78.0	4.0^{+}	1.61	92.3	95.0	-2.7	1.61
Trp	88.5	89.2	-0.7	1.28	94.2	96.1	-1.9	1.27

Table 3.6 (cont.)

Val	86.2	84.1	2.1	1.13	93.5	96.0	-2.5^{+}	1.13
Dispensable AA								
Ala	86.9	81.5	5.4*	1.68	97.8	98.7	-0.9	1.68
Asp	83.9	80.1	3.8*	1.36	92.7	94.9	-2.2	1.36
Cys	74.2	71.5	2.6	2.79	89.1	95.9	-6.8^{+}	2.79
Glu	90.7	90.6	0.1	0.61	94.1	96.1	-2.0*	0.61
Ser	81.1	80.8	0.4	1.10	88.5	93.0	-4.5**	1.10
Tyr	92.1	87.9	4.1**	0.75	97.3	97.9	-0.7	0.75

[1] Values are means and pooled SEMs. Labeled means in a row differ if *Measured vs. predicted, $P \leq 0.05$ or **Measured vs. predicted, $P \leq 0.01$; Or tend to differ if $^{+}$Measured vs. predicted, $0.05 < P \leq 0.10$.

[2] n indicates the number of replicates for each item within each treatment.

Table 3.7. Measured and predicted values for apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in the quick oats-dry milk meal-based diet fed to growing pigs[1]

	AID				SID			
Item, %	Measured	Predicted	Difference	SEM	Measured	Predicted	Difference	SEM
n^2	5	5			5	5		
CP	85.0	84.1	0.6	0.69	96.9	96.4	0.2	0.69
Indispensable AA								
Arg	87.6	88.5	-1.2	0.76	98.0	99.1	-1.4	0.76
His	88.5	88.8	-0.4	0.65	93.6	93.9	-0.5	0.65
Ile	87.1	86.6	0.4	0.62	92.1	91.7	0.3	0.62
Leu	89.8	89.9	-0.2	0.59	93.9	94.0	-0.2	0.59
Lys	87.3	87.1	-0.2	0.86	91.8	91.6	-0.1	0.86
Met	91.8	91.6	0.1	0.56	94.7	94.5	0.1	0.56
Phe	89.1	89.5	-0.5	0.67	93.1	93.5	-0.5	0.67
Thr	81.9	81.4	0.3	0.77	91.3	90.7	0.4	0.77
Trp	87.7	86.5	1.0	0.64	92.8	91.6	0.9	0.64

Table 3.7 (cont.)

Val	85.9	85.2	0.6	0.61	92.3	91.6	0.5	0.60
Dispensable AA								
Ala	81.9	81.1	0.5	1.47	92.7	92.2	0.3	1.47
Asp	85.2	85.0	0.0	0.95	92.0	91.9	-0.1	0.95
Cys	85.2	83.7	1.2	0.62	92.6	91.6	0.7	0.62
Glu	91.7	91.5	0.1	0.45	94.6	94.4	0.1	0.45
Ser	85.7	84.6	1.0^{+}	0.46	92.3	91.2	1.0^{+}	0.46
Tyr	88.7	89.3	-0.7	0.49	93.7	94.2	-0.5	0.49

[1] Values are means and pooled SEMs. Labeled means in a row differ if *Measured vs. predicted, $P \leq 0.05$ or **Measured vs. predicted, $P \leq 0.01$; Or tend to differ if $^{+}$Measured vs. predicted, $0.05 < P \leq 0.10$.

[2] n indicates the number of replicates for each item within each treatment.

Table 3.8. Digestible indispensable amino acid score (DIAAS) for the ingredients as measured in growing pigs[1]

Item	Corn flakes	Quick oats	Dry milk	SEM	*P*-value
n[2]	6	6	6		
Child (6 mo to 3yr)[3]					
DIAA reference ratio					
His	1.27	0.95	1.40		
Ile	1.19	1.03	1.62		
Leu	2.31	0.96	1.47		
Lys	0.16	0.57	1.38		
SAA	1.32	1.51	1.22		
AAA	1.88	1.45	1.87		
Thr	0.98	0.84	1.29		
Trp	0.55	1.09	1.66		
Val	1.05	1.00	1.44		
DIAAS[4], %	16^c (Lys)	57^b (Lys)	123^a (SAA)	1.13	<0.001
Older child, adolescent, adult[5]					

Table 3.8 (cont.)

DIAA reference ratio					
His	1.59	1.18	1.75		
Ile	1.27	1.10	1.72		
Leu	2.50	1.03	1.59		
Lys	0.19	0.67	1.64		
SAA	1.56	1.77	1.44		
AAA	2.38	1.84	2.38		
Thr	1.21	1.04	1.61		
Trp	0.71	1.40	2.14		
Val	1.13	1.07	1.55		
DIAAS^{4}, %	19^{c} (Lys)	67^{b} (Lys)	144^{a} (SAA)	1.31	<0.001

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[1] Values are means and pooled SEMs. Labeled means in a row without a common superscript letter differ, $P < 0.05$. AAA, aromatic amino acid; DIAA, digestible indispensable amino acid; SAA, sulfur amino acid.

[2] n indicates the number of replicates for each item within each treatment.

Table 3.8 (cont.)

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[3] DIAA reference ratios and DIAAS were calculated using the recommended AA scoring pattern for a child (6 months to 3 years). The indispensable AA reference patterns are expressed as mg AA/g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; SAA, 27; AAA, 52; Thr, 31; Trp, 8.5; Val, 43 (FAO, 2013).

[4] First-limiting AA is in parentheses.

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

Table 3.9. Measured and predicted values for digestible indispensable amino acid score (DIAAS) in combined meals of corn flakes-dry milk or quick oats-dry milk fed to growing pigs[1]

	Corn flakes-dry milk				Quick oats-dry milk			
Item	Measured	Predicted	Difference	SEM	Measured	Predicted	Difference	SEM
n[2]	6	6			5	5		
Child (6 mo to 3yr)[3]								
DIAA reference ratio								
His	1.34	1.36	-0.02	0.02	1.16	1.16	-0.01	0.01
Ile	1.43	1.48	-0.05*	0.01	1.31	1.30	0.00	0.01
Leu	1.70	1.73	-0.02$^+$	0.01	1.20	1.20	0.00	0.01
Lys	1.00	1.01	-0.01	0.01	0.95	0.95	0.00	0.01
SAA	1.22	1.26	-0.03$^+$	0.02	1.38	1.38	0.00	0.01
AAA	1.86	1.87	-0.02	0.01	1.64	1.65	-0.01	0.01
Thr	1.16	1.20	-0.03	0.02	1.06	1.05	0.00	0.01
Trp	1.30	1.32	-0.03	0.02	1.37	1.36	0.01	0.01
Val	1.29	1.32	-0.04$^+$	0.02	1.21	1.21	0.01	0.01

Table 3.9 (cont.)

DIAAS[4], %	100 (Lys)	101 (Lys)	-0.96	1.27	95 (Lys)	95 (Lys)	-0.15	0.89
Older child, adolescent, adult[5]								
DIAA reference ratio								
His	1.68	1.70	-0.03	0.02	1.45	1.45	-0.01	0.01
Ile	1.53	1.58	-0.06*	0.01	1.40	1.39	0.00	0.01
Leu	1.84	1.87	-0.03^{+}	0.01	1.29	1.30	0.00	0.01
Lys	1.19	1.20	-0.01	0.02	1.13	1.13	0.00	0.01
SAA	1.44	1.47	-0.04^{+}	0.02	1.63	1.62	0.00	0.01
AAA	2.35	2.38	-0.02	0.02	2.08	2.09	-0.01	0.01
Thr	1.44	1.48	-0.04	0.03	1.31	1.31	0.00	0.01
Trp	1.67	1.70	-0.03	0.02	1.76	1.75	0.02	0.01
Val	1.38	1.42	-0.04^{+}	0.02	1.30	1.30	0.01	0.01
DIAAS[4], %	119 (Lys)	120 (Lys)	-1.14	1.50	113 (Lys)	113 (Lys)	-0.17	1.06

[1] Values are means and pooled SEMs. Labeled means in a row differ if *Measured vs. predicted, $P \leq 0.05$ or **Measured vs. predicted, $P \leq 0.01$; Or tend to differ if $^{+}$Measured vs. predicted, $0.05 < P \leq 0.10$. AAA, aromatic amino acid; DIAA, digestible indispensable amino acid; SAA, sulfur amino acid.

Table 3.9 (cont.)

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[2] n indicates the number of replicates for each item within each treatment.

[3] DIAA reference ratios and DIAAS were calculated using the recommended AA scoring pattern for a child (6 months to 3 years). The indispensable AA reference patterns are expressed as mg AA/g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; SAA, 27; AAA, 52; Thr, 31; Trp, 8.5; Val, 43 (FAO, 2013).

[4] First-limiting AA is in parentheses.

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

Literature Cited

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

Ahn, J. Y., Kil, D. Y., Kong, C., and Kim, B. G. 2014. Comparison of oven-drying methods for determination of moisture content in feed ingredients. Asian-Australas. J. Anim. Sci. 27:1615-1622. doi: 10.5713/ajas.2014.14305

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

Cervantes-Pahm, S. K., and Stein, H. H. 2008. Effect of dietary soybean oil and soybean protein concentration on the concentration of digestible amino acids in soybean products fed to growing pigs. J. Anim. Sci. 86:1841-1849. doi:10.2527/jas.2007-0721

Cervantes-Pahm, S. K., Liu, Y., and Stein, H. H. 2014. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br. J. Nutr. 111:1663-1672. doi:10.1017/S0007114513004273

Finot, P. A. 1981. The extent of the Maillard reaction during the processing of milk. Prog. Food Nutr. Sci. 5:345-355.

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. Rome.

FAO. 2014. Research approaches and methods for evaluating the protein quality of human foods. Report of an FAO expert working group. Rome.

Han, F., Han, F., Wang, Y., Fan, L., Song, G., Chen, X., Jiang, P., Miao, H., and Han, Y. 2019. Digestible indispensable amino acid scores of nine cooked cereal grains. Br. J. Nutr. 121:30-41. doi:10.1017/S0007114518003033

Kim, B. G., Kil, D. Y., Zhang, Y., and Stein, H. H. 2012. Concentrations of analyzed or reactive lysine, but not crude protein, may predict the concentration of digestible lysine in distillers dried grains with solubles fed to pigs. J. Anim. Sci. 90:3798-3808. doi:10.2527/jas.2011-4692

Kwon, W. B., Touchette, K. J., Simongiovanni, A., Syriopoulos, K., Wessels, A., and Stein, H. H. 2019. 120 Effects of dietary leucine concentration on branched-chain amino acid metabolism in growing pigs. J. Anim. Sci. 97:65-66. doi:10.1093/jas/skz122.121

Mathai, J. K., Liu, Y., and Stein, H. H. 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

Myers, W. D., Ludden, P. A., Nayigihugu, V., and Hess, B. W. 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

Rafii, M., Elango, R., Ball, R. O., Pencharz, P. B., and Courtney-Martin, G. 2018. Metabolic availability of the limiting amino acids lysine and tryptophan in cooked white African cornmeal assessed in healthy young men using the indicator amino acid oxidation technique. J. Nutr. 148:917-924. doi:10.1093/jn/nxy039

Rutherfurd, S. M., Fanning, A. C., Miller, B. J., and Moughan, P. J. 2014. 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

Schwartz, M. B., Vartanian, L. R., Wharton, C. M., and Brownell, K. D. 2008. Examining the nutritional quality of breakfast cereals marketed to children. J. Acad. Nutr. Diet. 108:702-705. doi:10.1016/j.jada.2008.01.003

Shewry, P. R. 2007. Improving the protein content and composition of cereal grain. J. Cereal Sci. 46:239-250. doi:10.1016/j.jcs.2007.06.006

Shivakumar, N., Kashyap, S., Kishore, S., Thomas, T., Varkey, A., Devi, S., Preston, T., Jahoor, F., Sheshshayee, M. S., and Kurpad, A. V. 2019. Protein-quality evaluation of complementary foods in Indian children. Am. J. Clin. Nutr. 109:1319-1327. doi:10.1093/ajcn/nqy265

Stein, H. H., Pedersen, C., Wirt, A. R., and Bohlke, R. A. 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., Seve, B., Fuller, M. F., Moughan, P. J., and de Lange, C. F. M. 2007. 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

Stein, H. H., Shipley, C. F., and Easter, R. A. 1998. Technical note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433-1436. doi:10.2527/1998.7651433x

US Department of Agriculture, Agricultural Research Service. 2020. USDA Food Composition Database. https://ndb.nal.usda.gov (accessed June 2020).

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.

Xue, P. C., Ragland, D., and Adeola, O. 2014. Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. J. Anim. Sci. 92:3937-3944. doi:10.2527/jas.2014-7815

CHAPTER 4: DIGESTIBLE INDISPENSABLE AMINO ACID SCORE (DIAAS) IS GREATER IN ANIMAL-BASED BURGERS THAN IN PLANT-BASED BURGERS IF DETERMINED IN PIGS[2]

Abstract

The objective of this experiment was to determine digestible indispensable amino acid score (DIAAS) for animal- and plant-based burgers and test the hypothesis that DIAAS calculated for a burger and a burger bun is additive in a combined meal. Ten ileal cannulated gilts were fed experimental diets for six 9-d periods with ileal digesta being collected on d 8 and 9 of each period. Six diets contained a burger (i.e., 80% lean beef, 93% lean beef, 80% lean pork, Impossible Burger, or Beyond Burger) or a burger bun as the sole source of crude protein and amino acids. Three additional diets were based on a combination of the bun and 80% beef, pork, or Impossible Burger. A nitrogen-free diet was also used. The DIAAS for all ingredients and mixed meals was calculated for children from 6 months to 3 years and for individuals older than 3 years, and DIAAS for combined meals was predicted from individual ingredient DIAAS. The 93% lean beef and the pork burgers had greater ($P < 0.05$) DIAAS than the plant-based burgers for both age groups. The 80% lean beef burger had greater ($P < 0.05$) DIAAS than the plant burgers for children from 6 months to 3 years, and greater ($P < 0.05$) DIAAS than the Beyond Burger for individuals older than 3 years. There were no differences between the measured and predicted DIAAS. The protein quality of animal-based burgers is greater than that of plant-based

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[2] Material from: Natalia S. Fanelli et al., Digestible indispensable amino acid scores (DIAAS) for beef and pork burgers are higher than those for plant-based burgers, European Journal of Nutrition, published 2021, publisher: Springer. The copyright owner has provided permission to reprint.

burgers. However, for individuals older than 3 years, the Impossible Burger has comparable protein quality to the 80% lean beef burger. The DIAAS obtained from individual foods is additive in mixed meals.

Key words: amino acid digestibility, animal-based meat, digestible indispensable amino acid score, plant-based meat, protein quality.

Abbreviations: AA, amino acids; AAA, aromatic amino acids; AEE, acid hydrolyzed ether extract; AID, apparent ileal digestibility; Asx, sum of asparagine and aspartic acid; CP, crude protein; DIAA, digestible indispensable amino acids; DIAAS, digestible indispensable amino acid score; Glx, sum of glutamine and glutamic acid; SAA, sulfur amino acids; SE, standard error; SID, standardized ileal digestibility.

Introduction

In the US and most other developed countries, animal-based proteins provide a significant portion of the human diet (Daniel et al., 2011). When income is available, the demand for meat in developing countries continues to increase due to increased animal protein consumption; however, the increased supply is limited to specific countries and areas of Asia and South America, and does not occur in African countries (Speedy, 2003). On average, European citizen have an annual consumption of 22 kg of animal-based proteins and 16 kg of plant-based proteins (FAOSTAT, 2018). However, it is expected that animal protein consumption in Europe and North America will begin to decline by 2035 (Witte et al., 2021).

Plant-based proteins are available in the food supply chain and have become accepted as having an appearance, texture, and taste that is close to that of animal products (Ruby, 2012). The trend towards consuming more plant protein is also driven by concerns about animal welfare, human health, environmental impacts of animal production, religious practices, or personal preferences (Beardsworth and Keil, 1991). Therefore, there has been an interest in the development of plant-based proteins. Examples of these proteins include the Impossible Burger and the Beyond Burger, which are plant-based burgers made primarily from soy and pea protein, respectively.

The digestible indispensable amino acid score (**DIAAS**) has been recommended as the best method to determine protein quality in human foods. This method allows for calculation of the protein value of both individual ingredients and mixed meals consisting of several proteins (FAO, 2013). Values for DIAAS obtained in milk and breakfast cereals are additive in mixed meals (Fanelli et al., 2021), and the principle of additivity is believed to be applicable to all types of combined meals, but additional research to demonstrate this is needed. Therefore, the objectives of this experiment were to determine DIAAS values for animal and plant-based burgers and to test the hypothesis that DIAAS calculated for a burger and a burger bun are additive in a combined meal.

Materials and Methods

Preparation of ingredients and diets

Three animal-based burgers (80% lean beef, 93% lean beef, and 80% lean pork), two plant-based burgers (Impossible Burger and Beyond Burger), and one burger bun were included in the experiment (Table 4.1). Burgers were made of a round and flat patty of ingredients presented in Table 4.2. The ground beef and the plant-based products were purchased from Sysco Wholesale Company, Denver, CO, whereas the ground pork was purchased from Walmart Retail Company, Fort Collins, CO. All products were purchased in bulk, then formed into patties using a patty forming machine (Formax F6, Mokena, IL, USA), and cooked in a smokehouse (Enviro-Pak: CVU-650E, Clackamas, OR) at 157 °C and 20% humidity to an internal temperature of 71 °C as recommended (USDA-FSIS, 2019) at the Global Food Innovation Center at the Colorado State University, Fort Collins, CO. The burgers were allowed to cool for 30 min before being vacuum-packed and kept at − 20 °C until shipment to the University of Illinois, Urbana, IL, where the digestibility trial was conducted. The 80% and 93% lean meat burgers were chosen, because the 80% lean is most commonly consumed in restaurants and fast food establishments, whereas the 93% lean is a representative sample of leaner ground beef that many consumers select in grocery stores. The burger bun was produced at the Food Science and Human Nutrition Pilot Plant at the University of Illinois, Urbana, IL. Buns were baked at approximately 177 °C for 13–14 min using a turbo-flow gas convection oven (Imperial ICVG-2, Alton, IL, USA).

Each of the five burgers and the burger bun were included in one diet as the sole source of crude protein (**CP**) and amino acids (**AA**). Other ingredients in these diets included corn starch, solka floc, soy oil, and sucrose. Three additional diets were prepared by combining one patty (113g) of burger (i.e., 80% lean beef, pork, or Impossible Burger) and a burger bun (90g). A nitrogen-free diet was also prepared and used to measure basal endogenous losses of CP and AA to enable calculation of standardized ileal digestibility (**SID**) of CP and AA. Thus, a total of 10 diets were used in this experiment (Tables 4.3 and 4.4). Despite the recommended dietary protein content (dry matter basis) of 100g/kg (FAO, 2014, Hodgkinson et al., 2020), all diets were formulated to contain 150g/kg crude protein (dry matter basis) to feed diets closer to the AA requirements of growing pigs. For all diets, premix mixtures containing corn starch, solka floc, soy oil, sucrose, titanium dioxide, vitamins, and minerals were prepared to meet or exceed current nutrient requirement estimates for swine (NRC, 2012). With the exception of the nitrogen-free diet, the premix mixtures for all diets were mixed separately to allow for combination with ingredients before feeding. The titanium dioxide as indigestible marker was calculated in the premix mixtures to result in 0.60% titanium dioxide in the final diets. The burger bun dough also contained 0.60% titanium dioxide.

Ethical considerations

The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois before the research was conducted. All procedures performed by the investigators and farm staff were in accordance with the approved protocol.

Animals, housing, and feeding

Ten gilts (offspring of line 359 males mated to Camborough females; PIC, Henderson, TN, U.S.) with an initial average body weight of 24.6 ± 1.3 kg were equipped with a T-cannula in the distal ileum (Stein et al., 1998; Hodgkinson et al., 2020). Following surgery, pigs were allowed 7 days to recuperate and a grower diet based on corn and soybean meal was fed during this time. Pigs were then randomly allotted to a 10 × 6 Youden square design with 10 diets and six 9-day periods. No pig received the same diet more than once during the experiment and there were, therefore, six replicate pigs per treatment, which is the recommended number of pigs to have sufficient power of DIAAS experiments (FAO, 2014, Hodgkinson et al., 2020). All pigs were housed in individual pens (1.5 × 2.5 m) in an environmentally controlled room. Each pen had smooth sides and partially slatted floors. A nipple drinker and a feeder were also installed in each pen.

All pigs were fed their assigned diets in a daily amount equivalent to 4% of body weight at the start of each experimental period. Daily feed allowances were provided in two equal meals at 0800 and 1700 h, and the amount of feed supplied was recorded daily. Prior to feeding, the burger bun and all burgers were sliced into 2 cm pieces using a chopper slicer before being mixed with their respective premix mixture to ensure homogeneity with the indigestible marker. Although food ingredients and combinations of burger and bun could not be fed whole due to the need for an even marker mixing, they were generally served in a human-like format. Fresh water was available at all times. Environmental enrichment was ensured by providing toys to the pigs to reduce anxiety, stress, and abnormal repetitive behavior. Pig weights were recorded at the beginning of each experimental period to calculate feed allowance during the following period.

Sample collection

Although it is recognized that 7 day experimental periods are most often used (FAO, 2014), for practical reasons, experimental periods in this experiment were 9 days with the initial 7 days being the adaptation to each diet. Ileal digesta were collected for 9 h on days 8 and 9 following standard procedures with slight modifications (Stein et al., 1998; Hodgkinson et al., 2020). In short, a plastic bag was attached to the cannula barrel, and digesta flowing into the bag were collected. Bags were removed when filled with ileal digesta, or at least once every 30 minutes, and immediately frozen at – 20°C to prevent bacterial degradation of AA in the digesta. Cleaning of cannulas was completed every 24 hours (Hodgkinson et al., 2020). On the completion of one experimental period, animals were deprived of feed overnight, and in the following morning, a new experimental diet was offered. All pigs remained healthy during the experimental period and had a final average body weight of 53.4 ± 6.6 kg.

Chemical analysis

A sample of each food ingredient and of each diet was collected for chemical analysis. At the end of the experiment, ileal digesta were thawed, homogenized for each animal and diet, and a sub-sample was lyophilized and ground through a 0.5 mm screen prior to chemical analysis. Sub-samples of ingredients and diets were also lyophilized and ground through a 0.5 mm screen before analysis. All ingredients, diets, and ileal digesta samples were analyzed for dry matter [Method 927.05 (AOAC, 2007)] and for N by combustion [Method 990.0 (AOAC, 2007)] using a LECO analyzer (Model FP628, LECO Corp., Saint Joseph, MI, USA). Amino acids were analyzed on a Hitachi Amino Acid Analyzer (Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA, USA) after hydrolysis with 6*N* HCl for 24 h at 110°C [Method 982.30 E a, b, and c (AOAC, 2007)]. Crude protein was calculated as N × 6.25. Diets and ingredients samples were also analyzed for ash [Method 942.05; 10 (AOAC, 2007)]. Ingredients were analyzed for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments Co., Moline, IL, USA) with benzoic acid as the standard for calibration, and acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3*N* HCl (Model Ankom^{HCl}, Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (Model Ankom^{XT15}, Ankom Technology, Macedon, NY, USA). Calcium and phosphorus were also determined in all ingredients [Method 985.01 a, b, and c (AOAC, 2007)] using inductively coupled plasma-optical emission spectrometry (ICP-OES; Model Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600°C for 4 h [Method 942.05; 10 (AOAC, 2007)] and wet digestion with nitric acids [method 3050 B (U.S. Environmental Protection Agency, 2000)]. Titanium was analyzed for all diets and ileal digesta samples following the procedure of Myers et al. (2004).

Calculations

The apparent ileal digestibility (**AID**) of AA in each diet was calculated using the following equation (Stein et al., 2007):

$$\text{AID} = [1 - [(\text{AA}_{\text{digesta}}/\text{AA}_{\text{feed}}) \times (\text{Ti}_{\text{feed}}/\text{Ti}_{\text{digesta}})] \times 100,$$

where AID is the AID value of an AA (%), AAd is the concentration of that AA in the ileal digesta (dry matter), AAf is the AA concentration of that AA in the feed (dry matter), Tif is the titanium concentration in the feed (dry matter), and Tid is the titanium concentration in the ileal digesta (dry matter). The AID for CP was also calculated using this equation.

The basal endogenous flow to the distal ileum of each AA was determined based on the flow obtained after feeding the nitrogen-free diet using the following equation (Stein et al., 2007):

$$\text{Basal endogenous loss} = [\text{AA}_{\text{digesta}} \times (\text{Ti}_{\text{feed}}/\text{Ti}_{\text{digesta}})],$$

where the basal endogenous loss of each AA is determined in mg per kg dry matter intake. The basal endogenous loss of CP was determined using the same equation.

By correcting the AID for the basal endogenous loss of CP or AA, SID was calculated using the following equation (Stein et al., 2007):

$$\text{SID} = [(\text{AID} + \text{basal endogenous loss})/\ \text{AA}_{\text{feed}}],$$

where SID is the SID value of CP or AA (%).

The predicted AID of AA in the combined diet containing each burger and burger bun was calculated using the following equation (Stein et al., 2005):

$$\text{AID}_{\text{predicted}} = [(\text{AA}_{\text{burger}} \times \text{AID}_{\text{burger}}) + (\text{AA}_{\text{bun}} \times \text{AID}_{\text{bun}})]/(\text{AA}_{\text{burger}} + \text{AA}_{\text{bun}}),$$

where $\text{AID}_{\text{predicted}}$ (%) is the predicted AID for an AA in the mixed diet; $\text{AA}_{\text{burger}}$ and AA_{bun} are the concentrations (%) of that AA contributed by each burger and burger bun, respectively, which were calculated by multiplying the concentration of that AA (%) in the ingredient by the proportion (%) of the ingredient in the mixed diet; AID_{burger} and AID_{bun} are the determined AID (%) of the AA in each burger and burger bun, respectively. The predicted AID of CP and the SID of CP and all AA in the combined diets containing each burger and burger bun were calculated using the same equation.

The digestible indispensable amino acid (**DIAA**) reference ratios as well as DIAAS values for each protein or mixed diet were calculated as previously explained (Fanelli et al., 2021) and recommended by the Food and Agricultural Organization (FAO, 2013). Separate reference ratios were calculated for children from 6 months to 3 years, and for older children, adolescents, and adults.

Statistical analysis

At the conclusion of the experiment, normality of data was verified by generating studentized residuals from each analysis. Outliers were then identified and removed until the Shapiro-Wilk test reached $P < 0.05$ and studentized residuals were within ± 3 SD. Following this procedure, data were analyzed using the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC) in a randomized complete block design. The pig was the experimental unit for all analyses. Diet was the main effect and pig and period were random effects in the statistical model determining differences in AID, SID, and DIAAS among foods. Treatment means were calculated using the LSMEANS statement, and if significant, means were separated using the Student's *t*-test within the MIXED procedure. The LSMEANS were reported with corresponding standard errors (**SE**). Within each of the three combined meals, the Student's *t*-test was used to test the null hypothesis that the difference between the measured and predicted AID or SID of CP and AA, as well as values for DIAAS for the mixed diets, was equal to 0. Significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Pigs eating the animal-based burger diets and all three combinations of burgers and bun had an average daily gain between 0.70 and 0.90 kg/day. However, pigs eating the Impossible Burger, the Beyond Burger, and the burger bun diets had an average daily gain of 0.60, 0.40, and 0.20 kg/day, respectively. According to analyzed values for titanium in diets, calculated total titanium recovery was 94% for the burger diets and the nitrogen-free diet, 92% for the combinations of pork or Impossible Burger and bun, 100% for the combination of 80% lean beef burger and bun, and 96% for the burger bun diet.

The AID of CP, and some AA was greater ($P < 0.05$) in the 93% lean beef burger and in the pork burger than in the 80% lean beef burger and the Beyond Burger or the Impossible Burger (Table 4.5). All burgers had greater ($P < 0.05$) AID for Lys compared with the burger bun. The SID of CP, His, Ile, Leu, Met, and Val was greater ($P < 0.05$) in the 93% lean beef, the pork, and the Impossible Burger than in the Beyond Burger, but the burger bun had lower ($P < 0.05$) SID of Lys compared with all burgers. The SID of Val and the sum of sum of asparagine and aspartic acid (**Asx**) was greater ($P < 0.05$) in the pork burger than in the 80% lean beef burger, the Beyond Burger, and the burger bun.

Differences between the measured and the predicted AID and SID in the combined meal of 80% lean beef burger and bun differed ($P < 0.05$) from zero only for Trp (Table 4.6). For the combination of pork burger and bun, the AID and SID differed ($P < 0.05$) from zero for Cys

(Table 4.7). For the combined meal of Impossible Burger and bun, no differences between the measured and the predicted values for AID or SID were observed (Table 4.8).

For children from 6 months to 3 years and for individuals older than 3 years, the 93% lean beef and pork burgers had greater ($P < 0.05$) DIAAS compared with the other burgers, whereas the Beyond Burger had the lowest ($P < 0.05$) DIAAS for all burgers (Table 4.9). The animal-based burgers had greater ($P < 0.05$) DIAAS than the plant-based burgers for children between 6 months and 3 years. However, for children older than 3 years, adolescents, and adults, no differences were observed between the 80% lean beef and Impossible Burger, but the 80% lean beef had greater ($P < 0.05$) DIAAS than the Beyond Burger. The burger bun had lower ($P < 0.05$) DIAAS compared with all burgers. There was no limiting AA (DIAAS > 100) for the animal-based burgers for children from 6 months to 3 years, but for both plant-based burgers, the first limiting AA was the sulfur amino acids (**SAA**). For older children, adolescents and adults, there was no limiting AA (DIAAS > 100) for the animal-based burgers or for the Impossible Burger, but for the Beyond Burger, the first limiting AA was SAA. For both age groups, Lys was the first limiting AA in the burger bun.

For children from 6 months to 3 years, Val was the first limiting AA for the measured values of the combined meal of 80% lean beef burger and bun, but Lys was the first limiting AA for the predicted values, and there was no limiting AA for the combination of pork burger and bun (Table 4.10). For individuals older than 3 years, there was no limiting AA for the animal burger and bun combinations. However, for the combination of Impossible Burger and bun, Lys was the first limiting AA for both age groups. Differences between the measured and the predicted reference ratio values for the combination of 80% lean beef burger and bun differed ($P < 0.05$) from zero for Trp for both age groups. Likewise, differences between the measured and the predicted values for the combination of pork burger and bun differed ($P < 0.05$) from zero for SAA. For the Impossible Burger and bun, no differences between the measured and the predicted reference ratio values were observed. Regardless of age group and burger-bun combination, there were no differences between the measured and the predicted DIAAS.

Discussion

In recent years, DIAAS for cereal grains (Cervantes-Pahm et al., 2014; Abelilla et al., 2018; Han et al., 2019), breakfast cereals (Rutherfurd et al., 2014; Fanelli et al., 2021), pulse crops (Rutherfurd et al., 2014; Mathai et al., 2017), soy protein (Rutherfurd et al., 2014; Mathai et al., 2017), pistachio nuts [Bailey and Stein, 2020], milk proteins (Rutherfurd et al., 2014; Mathai et al., 2017; Fanelli et al., 2021), and meats (Hodgkinson et al., 2018; Bailey et al., 2020b; Bailey et al., 2020c) have been published. All of these values are important to build the nutrient database required to formulate diets for humans based on digestibility of AA (Fanelli et al., 2021). However, because proteins are usually consumed in meals consisting of several food items, it is important to demonstrate that values for DIAAS are additive in combined meals, which allow for calculation of DIAAS in combinations of food ingredients with individually determined values for DIAAS. In addition to providing values for DIAAS in animal based and plant-based burgers, results of the present experiment also provide data to demonstrate the additivity of DIAAS values, thus demonstrating the applicability of the DIAAS concept in practical nutrition.

Nutrient compositions of the animal-based burgers were within the range of published values (USDA Database, 2019) for cooked ground meat. Likewise, nutrient compositions of both plant-based burgers were within the range of published values for burgers made from plants (USDA Database, 2020). The nutrient composition of the burger bun was also within the range of published values for branded burger buns (USDA Database, 2020). The AID and SID of CP and AA that were determined for the beef burgers and the pork burger were within the range of reported values for pork loin and grilled topside steak, respectively (Hodgkinson et al., 2018; Bailey et al., 2020c). The SID for the Impossible Burger and the Beyond Burger were in agreement with SID values for soy protein isolate and pea protein concentrate, respectively (Mathai et al., 2017).

Nutritional losses of essential amino acids as well as the formation of Maillard reaction products are undesirable effects of protein-carbohydrate complexes when processing foods in the presence of heat (Jaeger et al., 2010). The Maillard reaction results in reduced concentration and digestibility of Lys and sometimes other AA (González-Vega et al., 2011; Almeida et al., 2014). Because there is a high concentration of Thr in mucin, which is the major component of endogenous protein lost at the end of the small intestine (Stein et al., 1999), the AID of Thr is expected to be the lowest among all indispensable AA, which was also the case for all burgers. However, the burger bun had a lower AID of Lys than of Thr indicating that baking at 177˚C for 13 to 14 minutes may have caused heat damage to the bun, because temperature and time of processing have a significant influence on destruction of Lys (Jaeger et al., 2010; González-Vega et al., 2011). It is not uncommon that cooking or baking results in heat damage and therefore low SID of Lys (Rutherfurd et al., 2014; Fanelli et al., 2021). As a consequence, the SID of Lys is often low in meals prepared from different baked foods (Almeida et al., 2011; Casas et al., 2015).

The few differences observed between the measured and the predicted SID values of AA for the combination of animal-based burgers and bun, and none for the combination of Impossible Burger and bun, demonstrated that values based on SID generally were additive in the combined meals. This is in agreement with data reported for other mixed diets (Stein et al., 2005;

Xue et al., 2014; Fanelli et al., 2021). The reason measured values for AID of CP were not different from predicted values is that concentrations of CP in diets based on individual ingredients were not different from the CP of the combined meals. As a consequence, there was no underestimation of AID in individual ingredients and AID values were, therefore, as additive as values for SID (Stein et al., 2005). However, if at least one low-protein food is used in a mixed meal, it is more accurate to use SID values to predict the digestibility for CP and AA in mixed meals (Stein et al., 2005; Xue et al., 2014). The difference between measured and calculated AID and SID for Trp in the 80% lean beef burger-bun combination may be due to analytical inaccuracies for Trp resulting in very high measured values.

A few values for SID were above 100%, which is not biologically possible. Basal endogenous losses were determined using a N-free diet, which many have slightly overestimated ileal endogenous losses and thereby resulted in SID values above 100%. However, the SID of most AA in ingredients based on the nitrogen-free diet method is additive in mixed diets, and the use of a nitrogen-free diet is preferred over purified proteins to measure endogenous basal loss of AA because of its simplicity and the definition of basal endogenous loss of AA (FAO, 2014; Adeola et al., 2016). It has also been demonstrated that although at least 3 days of adaptation is required, there is no differences in basal endogenous losses measured on d 6 and 7 or on d 8 and 9 (Kim et al., 2020).

Results for DIAAS in the beef burgers are close to published values for DIAAS in processed bovine meat (Bailey et al., 2020b), and the observation that DIAAS was greater than 100 in the pork burger is also in agreement with published values for DIAAS in pork products (Bailey et al., 2020c). Likewise, the fact that DIAAS was less than 100 in the burger bun and in the plant burgers is in agreement with published DIAAS values for cereal grains (Cervantes-

Pahm et al., 2014; Han et al., 2019) and other plant-based proteins (Mathai et al., 2017). The observation that the animal-based burgers had DIAAS values greater than 100 with no limiting AA for both age groups, indicates that the animal burgers are "excellent" sources of protein (FAO, 2013). However, because the Impossible Burger had DIAAS greater than 75 and less than 100 for children less than 3 years old, this burger only qualifies as a "good" source of protein according to the Food and Agricultural Organization (FAO, 2013). In contrast, because the Impossible Burger had DIAAS greater than 100 for individuals older than 3 years, it is an "excellent" source of protein for this age group (FAO, 2013). For children older than 3 years, adolescents, and adults, the Beyond Burger qualifies as a "good" source of protein (FAO, 2013), but due to the low DIAAS in Beyond Burger for children from 6 months to 3 years, no protein claim can be made for this age group (FAO, 2013).

The Impossible Burger was produced using soy protein concentrate as the main source of protein with coconut oil and sunflower oil being the sources of oil. The Beyond Burger was based on pea protein isolate as the main source of protein whereas expeller-pressed canola oil and refined coconut oil were the sources of oil. It is, therefore, not surprising that DIAAS for the Impossible Burger was greater than that of the Beyond Burger, because soy protein concentrate has a greater DIAAS than pea protein isolate (Mathai et al., 2017).

The observation that the animal-based burger and bun combinations had DIAAS very close to or equal to 100 for children from 6 months to 3 years, and greater than 100 for individuals older than 3 years, indicates that the high protein quality in animal meat can compensate for the low protein quality in the burger bun. As a consequence, the combination of animal burger and bun provides a balanced ratio of indispensable AA. The low DIAAS in the combination of Impossible Burger and bun for both age groups, and with Lys as first limiting

AA, demonstrates that there is insufficient Lys in the Impossible Burger to compensate for the low concentration of Lys in the burger bun. The ingredients used to produce the burger bun were grain-based ingredients, and cereal grains always have Lys as first limiting AA (Cervantes-Pahm et al., 2014; Han et al., 2019). In addition to Lys, Leu, SAA, and Thr, were also limiting in the Impossible Burger-bun combination for children from 6 months to 3 years, but for individuals older than 3 years, only Lys was limiting. Thus, to meet AA requirements for children from 6 months to 3 years, the Impossible Burger-bun combination needs to be complemented by another high Lys ingredient that also provides some Leu, SAA, and Thr.

With the exception of the tendency for a greater predicted DIAAS in the pork burger-bun combination compared with the measured DIAAS for individuals older than 3 years, no differences between the measured and the predicted DIAAS were observed. This demonstrates that by measuring DIAAS in individual food ingredients, DIAAS in combined meals can be predicted with reasonable accuracy. This is an important observation because it will not be possible to measure DIAAS in all possible combinations of foods. However, if DIAAS is measured in individual ingredients, the protein quality of mixed meals or combinations of foods can be predicted as demonstrated in this experiment. This is in agreement with recent data demonstrating that DIAAS in milk and breakfast cereals can be used to accurately predict DIAAS in breakfast cereal-milk combinations (Fanelli et al., 2021).

Ideally, humans should be used to determine DIAAS in human foods (FAO, 2014). However, collecting digesta from the distal ileum in humans is invasive and expensive, and although methods that do not require digesta sampling have been developed, they are not suitable for routine food evaluation (FAO, 2014; Hodgkinson et al., 2020). As a result, the growing pig, is recommended for determining DIAAS in human foods (FAO, 2013). The advantage of using a pig model over clinical trials is that it is a rapid method and there is already a large dataset with values for SID of AA in foods that can be translated to the human dataset with a subsequent calculation of DIAAS (Hodgkinson et al., 2020). Other benefits of this model include that effects of processing on DIAAS can be investigated (Hodgkinson et al., 2020; Fanelli et al., 2021), and there are a number of laboratories around the world that are capable of determining digestibility of AA by pigs (FAO, 2014). However, one of the limitations of using an animal model is that, despite well-described similarities in AA digestibility between growing pigs and humans, protein digestibility and nutrient absorption are unlikely to be identical, and data from pigs do not account for effects of different social and environmental conditions (FAO, 2014).

Conclusion

Results of this research demonstrated that DIAAS in beef and pork burgers are greater than DIAAS in plant-based burgers. However, for individuals older than 3 years, the Impossible Burger had DIAAS that was not different from that in the 80% lean beef burger. The high DIAAS in beef and pork burgers makes it possible to compensate for the low protein quality in burger buns by combining beef or pork burgers with a burger bun. In contrast, the combination of the Impossible Burger and burger bun does not provide enough digestible AA to meet the requirements for individuals older than 6 months. Results also demonstrated that DIAAS in the combined meals of burger and burger bun can be predicted from the individual ingredient DIAAS values, and because most individuals eat meals that consist of several proteins, it is important that DIAAS of individual ingredients are additive in mixed meals. Therefore, if more data can be established for different individual food ingredients, it will be possible to predict DIAAS for different combinations of foods.

Tables

Table 4.1. Analyzed nutrient composition of food ingredients (as fed-basis)[1]

Item, %	80% lean beef	93% lean beef	Pork	Impossible Burger	Beyond Burger	Burger bun
Dry matter	44.52	41.34	40.53	44.14	47.36	70.64
Gross energy (kcal/kg)	3,220	2,720	2,875	2,572	3,203	3,355
CP	24.04	27.61	19.62	18.10	20.22	12.10
AEE	16.92	11.59	15.98	10.65	9.33	3.40
Ash	0.70	1.55	0.87	2.66	1.63	1.65
Minerals						
Calcium	0.01	0.01	0.01	0.21	0.09	0.05
Phosphorus	0.15	0.18	0.19	0.22	0.29	0.10
Indispensable AA						
His	0.65	0.85	0.62	0.42	0.50	0.24
Ile	1.02	1.34	0.90	0.87	1.00	0.45
Leu	1.73	2.20	1.48	1.35	1.69	0.78
Lys	1.79	2.32	1.55	1.02	1.36	0.28

Table 4.1 (cont.)

Met	0.54	0.72	0.49	0.19	0.26	0.18
Phe	0.93	1.14	0.78	0.93	1.16	0.59
Thr	0.92	1.19	0.83	0.68	0.75	0.32
Trp	0.25	0.33	0.23	0.23	0.18	0.13
Val	1.15	1.38	0.97	0.94	1.12	0.50
Total	8.98	11.47	7.85	6.63	8.02	3.47
Dispensable AA						
Ala	1.46	1.56	1.16	0.75	0.88	0.36
Arg	1.50	1.74	1.29	1.11	1.63	0.42
Asx	1.96	2.48	1.71	1.91	2.23	0.52
Cys	0.23	0.29	0.19	0.35	0.27	0.25
Glx	3.09	3.94	2.57	3.58	3.18	3.69
Gly	1.74	1.35	1.29	0.73	0.80	0.41
Pro	1.22	1.10	0.93	0.82	0.89	1.21
Ser	0.80	0.96	0.70	0.72	0.96	0.50
Tyr	0.80	1.01	0.71	0.68	0.78	0.33

Table 4.1 (cont.)

Total	12.80	14.43	10.55	10.65	11.62	7.69
Total AA	21.78	25.90	18.41	17.28	19.64	11.16

[1] AA, amino acids; AEE, acid hydrolyzed ether extract; Asx, sum of asparagine and aspartic acid; CP, crude protein; Glx, sum of glutamine and glutamic acid.

Table 4.2. Ingredient composition of burger products

Burger patty	Ingredients
80% lean beef[1]	Beef trimmings 80% lean, 20% fat
93% lean beef[1]	Beef trimmings 93% lean, 7% fat
80% lean pork[1]	Pork trimmings 80% lean, 20% fat
Impossible Burger[2]	Water, soy protein concentrate, coconut oil, sunflower oil, and natural flavors 2% or less of: potato protein, methylcellulose, yeast extract, cultured dextrose, food starch modified, soy leghemoglobin, salt, mixed tocopherols, soy protein isolate, vitamins and minerals [zinc gluconate, thiamine hydrochloride (vitamin B1), niacin, pyridoxine hydrochloride (vitamin B6), riboflavin (vitamin B2), and vitamin B12]
Beyond Burger[3]	Water, pea protein isolate, expeller-pressed canola oil, and refined coconut oil 2% or less of: cellulose from bamboo, methylcellulose, potato starch, natural flavors, maltodextrin, yeast extract, salt, sunflower oil, vegetable glycerin, dried yeast, gum arabic, citrus extract, ascorbic acid, beet juice extract, acetic acid, succinic acid, modified food starch, annatto, and minerals (calcium, iron, salt, and potassium chloride)

[1] No other ingredients or binders were included in the animal-based burger patties according to federal regulations (9 CFR § 319).

Table 4.2 (cont.)

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[2] Contains allium derivative. Flavor made from heme, via fermentation of genetically engineered yeast. Mixed tocopherols as antioxidant. Binders: Methylcellulose, food starch modified.

[3] All ingredients derivate from Non-GMO sources. Citrus extract to protect quality. Beet juice extract for flavor. Annatto for color. Ascorbic acid to maintain color. Binders: Methylcellulose, food starch modified.

Table 4.3. Ingredient composition of the 10 experimental diets (as fed-basis)[1]

Item, %	80% lean beef	93% lean beef	Pork	Impossible Burger	Beyond Burger	Burger bun	80% lean beef + bun	80% lean pork + bun	Impossible Burger + bun	Nitrogen-free
Burger	42.80	42.80	55.10	55.10	52.50	-	32.50	37.10	37.10	-
Burger bun	-	-	-	-	-	90.60	25.90	29.60	29.60	-
Corn starch	25.05	25.05	12.83	12.83	15.52	-	9.49	6.25	6.25	67.67
Solka floc	4.00	4.00	4.00	4.00	4.00	-	4.00	4.00	4.00	4.00
Soy oil	4.00	4.00	4.00	4.00	4.00	0.30	4.00	4.00	4.00	4.00
Monocalcium phosphate	1.40	1.40	1.25	1.25	1.30	1.40	1.40	1.30	1.30	1.70
Limestone	1.10	1.10	1.17	1.17	1.03	1.00	1.06	1.10	1.10	0.98
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Magnesium oxide	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Potassium carbonate	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sucrose	20.00	20.00	20.00	20.00	20.00	5.05	20.00	15.00	15.00	20.00
Titanium dioxide	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60

Table 4.3 (cont.)

Vitamin mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

[1] All diets, except the nitrogen-free, were formulated to contain approximately 15% CP (dry matter basis).

[2] 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 4.4. Analyzed nutrient composition of the 10 experimental diets (as fed-basis)[1]

Item, %	80% lean beef	93% lean beef	Pork	Impossible Burger	Beyond Burger	Burger bun	80% lean beef + bun	80% lean pork + bun	Impossible Burger + bun	Nitrogen-free
Dry matter	74.12	73.06	65.50	67.09	69.91	71.02	73.09	67.35	70.05	91.96
CP	9.68	12.08	10.19	9.63	10.29	10.57	8.52	10.56	10.36	0.42
Ash	3.10	3.28	3.23	3.78	3.44	3.70	3.92	3.82	3.83	4.09
Indispensable AA										
His	0.29	0.37	0.36	0.23	0.24	0.21	0.23	0.31	0.24	0.00
Ile	0.48	0.57	0.51	0.46	0.48	0.38	0.38	0.46	0.46	0.01
Leu	0.81	0.94	0.83	0.72	0.80	0.67	0.66	0.79	0.76	0.02
Lys	0.87	1.04	0.91	0.57	0.69	0.22	0.58	0.69	0.49	0.01
Met	0.23	0.29	0.26	0.11	0.12	0.13	0.19	0.23	0.13	0.01
Phe	0.43	0.49	0.44	0.50	0.56	0.50	0.39	0.46	0.54	0.02
Thr	0.43	0.50	0.46	0.36	0.35	0.28	0.33	0.40	0.36	0.01
Trp	0.09	0.15	0.12	0.12	0.10	0.11	0.10	0.13	0.13	0.02
Val	0.50	0.59	0.54	0.49	0.53	0.42	0.40	0.50	0.50	0.01

Table 4.4 (cont.)

Total	4.13	4.94	4.43	3.56	3.87	2.92	3.26	3.97	3.61	0.11
Dispensable AA										
Ala	0.59	0.69	0.64	0.41	0.43	0.31	0.47	0.57	0.40	0.01
Arg	0.62	0.72	0.67	0.59	0.76	0.33	0.48	0.59	0.55	0.01
Asx	0.92	1.07	0.97	1.03	1.07	0.43	0.67	0.82	0.88	0.02
Cys	0.10	0.12	0.11	0.23	0.13	0.20	0.11	0.14	0.24	0.00
Glx	1.50	1.78	1.55	2.09	1.60	3.14	1.73	2.03	2.65	0.02
Gly	0.54	0.59	0.67	0.39	0.38	0.35	0.53	0.67	0.41	0.01
Pro	0.44	0.49	0.50	0.46	0.43	1.04	0.60	0.71	0.71	0.02
Ser	0.34	0.39	0.36	0.40	0.42	0.42	0.33	0.41	0.44	0.01
Tyr	0.31	0.35	0.24	0.28	0.31	0.19	0.26	0.33	0.29	0.01
Total	5.36	6.20	5.71	5.88	5.53	6.41	5.18	6.27	6.57	0.11
Total AA	9.49	11.14	10.14	9.44	9.40	9.33	8.44	10.24	10.18	0.22

[1] AA, amino acids; Asx, sum of asparagine and aspartic acid; CP, crude protein; Glx, sum of glutamine and glutamic acid.

Table 4.5. Apparent ileal digestibility (AID) of crude protein (CP) and amino acids (AA) in food ingredients[1]

Item, %	80% lean beef	93% lean beef	Pork	Impossible Burger	Beyond Burger	Burger bun	SE	*P*-value
CP	73.3^{c}	85.8^{ab}	88.2^{a}	82.2^{abc}	73.2^{c}	79.0^{bc}	3.14	0.006
Indispensable AA								
His	87.5^{cd}	91.6^{ab}	93.7^{a}	89.1^{bc}	83.5^{e}	85.1^{de}	1.27	<0.001
Isle	88.8^{b}	91.5^{ab}	92.1^{a}	89.3^{ab}	85.4^{c}	85.3^{c}	1.05	0.0002
Leu	89.6^{bc}	92.4^{ab}	92.8^{a}	89.2^{c}	85.6^{d}	87.8^{cd}	1.03	0.0002
Lys	91.8^{a}	94.0^{a}	94.7^{a}	89.5^{a}	88.8^{a}	46.7^{b}	2.99	<0.001
Met	93.3^{a}	95.0^{a}	94.9^{a}	89.8^{b}	79.3^{c}	88.9^{b}	0.85	<0.001
Phe	87.4	90.5	91.2	90.7	87.9	89.7	1.14	0.100
Thr	78.9^{c}	85.0^{ab}	86.7^{a}	79.4^{bc}	74.1^{c}	73.4^{c}	2.08	0.0003
Trp	85.9^{d}	94.7^{ab}	96.6^{a}	92.8^{abc}	90.0^{bcd}	87.9^{cd}	1.92	0.001
Val	85.5^{bc}	89.6^{a}	90.7^{a}	87.4^{ab}	83.0^{c}	82.6^{c}	1.40	0.001
Mean	88.1^{c}	91.5^{ab}	92.4^{a}	88.4^{bc}	84.9^{d}	82.5^{d}	1.18	<0.001
Dispensable AA								

Table 4.5 (cont.)

Ala	83.9^{ab}	90.9^{a}	92.3^{a}	84.7^{ab}	76.5^{bc}	68.8^{c}	3.08	0.0002
Arg	84.5^{b}	92.7^{ab}	95.0^{a}	91.6^{ab}	87.7^{ab}	74.0^{c}	3.56	0.006
Cys	48.4^{c}	62.2^{bc}	67.3^{b}	70.8^{ab}	53.1^{c}	84.6^{a}	4.94	0.0004
Glx	88.9^{b}	92.8^{a}	93.6^{a}	92.8^{a}	87.3^{b}	95.1^{a}	1.08	0.0001
Gly	53.1^{c}	78.4^{ab}	87.9^{a}	70.9^{ab}	49.2^{c}	54.6^{c}	8.23	0.012
Ser	74.0^{b}	84.0^{a}	86.4^{a}	84.1^{a}	79.2^{ab}	83.5^{a}	2.69	0.030
Tyr	86.0^{ab}	89.8^{a}	87.9^{ab}	88.8^{ab}	85.5^{b}	79.9^{c}	1.44	0.001
Mean	80.9^{b}	88.9^{a}	91.1^{a}	86.7^{ab}	81.0^{b}	85.9^{ab}	2.09	0.007
Total AA	84.2^{b}	90.1^{a}	91.7^{a}	87.4^{ab}	82.6^{b}	84.7^{b}	1.65	0.003

[a,b,c,d] Means in a row without a common superscript letter differ $P < 0.05$. Values are means and pooled SEs, n = 5 for 93% lean beef, Impossible Burger, and burger bun, n = 6 for 80% lean beef, pork, and Beyond Burger.

[1] AA, amino acids; Asx, sum of asparagine and aspartic acid; CP, crude protein; Glx, sum of glutamine and glutamic acid; SE, standard error.

Table 4.6. Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in food ingredients[1]

Item, %	80% lean beef	93% lean beef	Pork	Impossible Burger	Beyond Burger	Burger bun	SE	*P*-value
CP	91.9^{bc}	100.5^{ab}	103.8^{a}	99.1^{ab}	89.7^{c}	95.2^{abc}	3.14	0.024
Indispensable AA								
His	93.1^{bc}	96.0^{ab}	97.7^{a}	95.6^{ab}	89.8^{c}	92.4^{bc}	1.27	0.002
Isle	93.8^{ab}	95.7^{a}	96.3^{a}	94.1^{ab}	90.1^{c}	91.3^{bc}	1.05	0.001
Leu	94.2^{ab}	96.2^{a}	96.8^{a}	93.9^{ab}	90.0^{c}	93.2^{b}	1.03	0.001
Lys	96.3^{a}	97.7^{a}	98.6^{a}	95.8^{a}	94.2^{a}	63.9^{b}	2.99	<0.001
Met	95.8^{a}	97.0^{a}	97.0^{a}	94.7^{ab}	83.9^{c}	93.0^{b}	0.85	<0.001
Phe	92.7	95.1	95.9	94.9	91.8	94.1	1.14	0.109
Thr	88.9^{bc}	93.4^{ab}	95.1^{a}	90.2^{abc}	85.8^{c}	88.3^{bc}	2.08	0.031
Trp	94.9^{b}	100.1^{a}	102.4^{a}	99.0^{ab}	97.8^{ab}	94.8^{b}	1.92	0.023
Val	92.1^{bcd}	95.2^{ab}	96.1^{a}	93.5^{abc}	88.9^{d}	90.2^{cd}	1.40	0.006
Mean	93.7^{bc}	96.1^{ab}	97.0^{a}	94.3^{ab}	90.5^{cd}	90.0^{d}	1.18	0.001
Dispensable AA								

Table 4.6 (cont.)

Ala	93.7^{abc}	99.2^{a}	100.2^{a}	97.4^{ab}	89.2^{bc}	86.4^{c}	3.08	0.023
Arg	97.4	103.8	105.5	103.9	97.7	97.1	3.56	0.322
Cys	62.9^{c}	73.7^{bc}	78.8^{ab}	76.6^{bc}	64.0^{c}	91.6^{a}	4.94	0.004
Glx	93.9^{bc}	97.0^{ab}	98.0^{a}	96.1^{ab}	91.8^{c}	97.4^{a}	1.08	0.002
Gly	83.8	105.9	109.7	109.3	89.7	100.2	8.23	0.149
Ser	86.6	94.7	96.9	93.9	88.9	93.3	2.69	0.084
Tyr	92.3^{ab}	95.3^{a}	95.0^{a}	95.2^{a}	91.5^{ab}	89.7^{b}	1.44	0.039
Mean	91.5^{b}	97.9^{a}	99.9^{a}	95.4^{ab}	90.6^{b}	95.2^{ab}	2.09	0.025
Total AA	92.5^{bc}	97.1^{ab}	98.5^{a}	95.0^{abc}	90.5^{c}	93.3^{bc}	1.65	0.017

[a,b,c,d] Means in a row without a common superscript letter differ $P < 0.05$. Values are means and pooled SEs, n = 5 for 93% lean beef, Impossible Burger, and burger bun, n = 6 for 80% lean beef, pork, and Beyond Burger.

[1] AA, amino acids; Asx, sum of asparagine and aspartic acid; CP, crude protein; Glx, sum of glutamine and glutamic acid; SE, standard error.

[2] The SID values were calculated by correcting values for basal ileal endogenous losses (g/kg dry matter intake): Crude protein 24.25, Arg 1.08, His 0.22, Ile 0.32, Leu 0.50, Lys 0.53, Met 0.08, Phe 0.31, Thr 0.58, Trp 0.11, Val 0.45, Ala 0.78, Asx 0.85, Cys 0.20, Glx 1.02, Ser 0.58, and Tyr 0.26.

Table 4.7. Measured and predicted values for apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in the 80% lean beef burger and bun meal[1,2]

	AID				SID			
Item, %	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE
CP	74.9	74.9	0.0	4.57	95.7	92.8	2.9	4.57
Indispensable AA								
His	86.8	86.9	-0.1	1.69	93.8	92.9	0.9	1.69
Ile	86.7	87.9	-1.2	1.28	93.0	93.2	-0.2	1.28
Leu	88.7	89.2	-0.5	1.13	94.3	93.9	0.3	1.13
Lys	87.5	86.9	0.6	1.53	94.2	92.8	1.4	1.53
Met	92.3	92.4	-0.1	0.70	95.3	95.2	0.0	0.70
Phe	87.5	88.1	-0.7	1.26	93.3	93.2	0.1	1.26
Thr	76.9	77.7	-0.9	2.67	89.8	88.8	1.0	2.67
Trp	94.5	86.5	8.0**	1.42	102.6	94.9	7.7**	1.42
Val	83.7	84.8	-1.1	1.67	91.8	91.6	0.2	1.67
Mean	86.6	86.8	-0.3	1.41	93.5	92.8	0.7	1.41

Table 4.7 (cont.)

Dispensable AA								
Ala	83.7	81.5	2.2	2.39	95.8	92.5	3.3	2.39
Arg	85.2	82.6	2.6	3.35	101.7	97.4	4.4	3.35
Asx	82.3	82.1	0.3	2.26	91.6	90.2	1.5	2.26
Cys	63.8	64.8	-1.1	3.59	76.8	75.9	0.8	3.59
Glx	92.0	91.9	0.2	1.05	96.4	95.6	0.8	1.05
Gly	67.5	53.4	14.2	7.07	98.3	86.3	11.9	7.07
Ser	78.9	77.1	1.7	3.04	91.7	88.8	2.9	3.04
Tyr	86.4	84.5	1.8	1.42	93.7	91.7	2.1	1.42
Mean	84.3	82.6	1.7	2.49	95.4	92.7	2.7	2.49
Total AA	85.2	84.3	0.9	2.03	94.6	92.7	1.9	2.03

[1] AA, amino acids; Asx, sum of asparagine and aspartic acid; CP, crude protein; Glx, sum of glutamine and glutamic acid; SE, standard error.

[2] Means in a row differ if *Measured vs. predicted $P \leq 0.05$, **Measured vs. predicted $P \leq 0.01$, or tend to differ if $^{+}$Measured vs. predicted $0.05 < P \leq 0.10$. Values are means and pooled SEs, n = 6.

Table 4.8. Measured and predicted values for apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in the pork burger and bun meal[1,2]

	AID				SID			
Item, %	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE
CP	78.1	86.1	-7.9	4.76	93.6	100.7	-7.1	4.76
Indispensable AA								
His	88.8	91.7	-2.9$^+$	1.38	93.6	96.5	-2.9$^+$	1.38
Ile	87.8	90.2	-2.4$^+$	1.10	92.6	94.9	-2.3$^+$	1.10
Leu	89.5	91.4	-1.9	0.97	93.8	95.7	-2.0	0.97
Lys	88.9	88.7	0.3	1.54	94.1	94.2	0.0	1.54
Met	92.9	93.6	-0.7	0.68	95.2	96.1	-0.9	0.68
Phe	88.4	90.6	-2.2$^+$	1.06	93.0	95.2	-2.2$^+$	1.06
Thr	79.3	83.6	-4.3$^+$	1.66	89.1	93.5	-4.3$^+$	1.66
Trp	94.2	93.9	0.3	1.55	99.8	100.0	-0.2	1.55
Val	85.6	88.4	-2.7$^+$	1.31	91.7	94.4	-2.7$^+$	1.31
Mean	87.9	89.9	-2.0	1.21	93.1	95.2	-2.1	1.21

Table 4.8 (cont.)

Dispensable AA								
Ala	84.7	87.7	-3.0	3.12	93.8	97.5	-3.7	3.12
Arg	84.8	90.7	-5.9	4.66	97.1	103.8	-6.6	4.66
Asx	84.1	87.2	-3.2	1.91	91.1	94.6	-3.5	1.91
Cys	68.3	76.2	-7.9*	2.06	77.7	85.3	-7.7*	2.06
Glx	92.3	94.4	-2.1	1.10	95.7	97.7	-2.0	1.10
Gly	72.4	81.1	-8.7	8.91	94.8	107.8	-13.0	8.91
Ser	81.4	85.3	-4.0	2.31	90.9	95.6	-4.7+	2.31
Tyr	87.9	85.8	2.1	1.18	93.2	93.6	-0.4	1.18
Mean	85.4	89.1	-3.7	2.81	93.9	98.1	-4.1	2.81
Total AA	86.5	89.4	-3.0	2.11	93.6	96.8	-3.3	2.11

[1] AA, amino acids; Asx, sum of asparagine and aspartic acid; CP, crude protein; Glx, sum of glutamine and glutamic acid; SE, standard error.

[2] Means in a row differ if *Measured vs. predicted $P \le 0.05$, **Measured vs. predicted $P \le 0.01$, or tend to differ if +Measured vs. predicted $0.05 < P \le 0.10$. Values are means and pooled SEs, n = 6.

Table 4.9. Measured and predicted values for apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in the Impossible Burger and bun meal[1,2]

	AID				SID			
Item, %	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE
CP	81.6	81.1	0.5	2.60	98.0	97.8	0.2	2.60
Indispensable AA								
His	86.5	87.9	-1.4	1.87	92.9	94.6	-1.7	1.87
Ile	86.8	88.1	-1.3	1.64	91.8	93.3	-1.4	1.64
Leu	87.8	88.8	-1.0	1.69	92.4	93.7	-1.2	1.69
Lys	84.2	81.8	2.4	2.03	91.8	90.1	1.7	2.03
Met	88.1	89.4	-1.2	1.45	92.5	94.0	-1.5	1.45
Phe	89.5	90.4	-0.9	1.43	93.6	94.6	-1.1	1.43
Thr	75.2	77.7	-2.5	3.21	86.7	89.7	-3.0	3.21
Trp	92.0	91.3	0.7	1.92	98.1	97.6	0.5	1.92
Val	84.7	86.0	-1.3	1.99	91.0	92.5	-1.6	1.99
Mean	85.8	86.7	-0.9	1.89	91.9	93.0	-1.2	1.89

Table 4.9 (cont.)

Dispensable AA								
Ala	81.6	80.3	1.3	2.34	95.2	94.3	0.8	2.34
Arg	89.4	87.5	1.9	1.54	103.3	102.3	1.0	1.55
Asx	79.2	80.6	-1.4	2.41	86.0	87.7	-1.7	2.41
Cys	71.3	75.8	-4.5	2.83	77.0	82.0	-4.9	2.83
Glx	93.3	93.8	-0.5	0.82	96.0	96.7	-0.7	0.82
Gly	70.1	65.8	4.2	3.97	108.5	106.5	2.0	3.97
Ser	83.1	83.9	-0.8	2.13	92.3	93.7	-1.4	2.13
Tyr	86.4	86.3	0.2	1.88	92.8	93.6	-0.8	1.88
Mean	86.4	86.4	0.0	1.61	94.8	95.3	-0.6	1.61
Total AA	86.2	86.5	-0.3	1.73	93.7	94.4	-0.7	1.73

[1] AA, amino acids; Asx, sum of asparagine and aspartic acid; CP, crude protein; Glx, sum of glutamine and glutamic acid; SE, standard error.

[2] Means in a row differ if *Measured vs. predicted $P \leq 0.05$, **Measured vs. predicted $P \leq 0.01$, or tend to differ if $^{+}$Measured vs. predicted $0.05 < P \leq 0.10$. Values are means and pooled SEs, n = 6.

Table 4.10. Digestible indispensable amino acids (DIAA) reference ratio and digestible indispensable amino acid score (DIAAS) in food ingredients as measured in growing pigs[1]

Item	80% lean beef	93% lean beef	Pork	Impossible Burger	Beyond Burger	Burger bun	SE	*P*-value
Child (6 months to 3 years)[2]								
DIAA reference ratio								
His	1.27	1.47	1.55	1.12	1.11	0.92		
Ile	1.24	1.45	1.38	1.41	1.40	1.06		
Leu	1.03	1.16	1.11	1.06	1.14	0.91		
Lys	1.25	1.44	1.36	0.95	1.11	0.26		
SAA	1.03	1.22	1.17	0.91	0.71	1.21		
AAA	1.28	1.42	1.39	1.62	1.69	1.35		
Thr	1.10	1.30	1.29	1.09	1.02	0.75		
Trp	1.16	1.41	1.42	1.45	1.05	1.20		
Val	1.02	1.11	1.11	1.13	1.14	0.87		
DIAAS[3], %	102^{b}	111^{a}	111^{a}	91^{c} (SAA)	71^{d} (SAA)	26^{e} (Lys)	2.36	<0.001

Table 4.10 (cont.)

Older child, adolescent, adult[2]								
DIAA reference ratio								
His	1.58	1.84	1.94	1.40	1.39	1.15		
Ile	1.33	1.54	1.47	1.51	1.49	1.13		
Leu	1.11	1.25	1.20	1.14	1.23	0.98		
Lys	1.49	1.71	1.62	1.13	1.32	0.31		
SAA	1.20	1.43	1.38	1.07	0.83	1.42		
AAA	1.62	1.81	1.77	2.06	2.15	1.72		
Thr	1.36	1.62	1.60	1.35	1.27	0.93		
Trp	1.49	1.81	1.83	1.87	1.35	1.54		
Val	1.10	1.19	1.19	1.21	1.23	0.93		
DIAAS[3], %	110^{b}	119^{a}	119^{a}	107^{b}	83^{c} (SAA)	31^{d} (Lys)	2.51	<0.001

[a,b,c,d,e] Means in a row without a common superscript letter differ *P* < 0.05. Values are means and pooled SEs, n = 5 for 93% lean beef, Impossible Burger, and burger bun, n = 6 for 80% lean beef, pork, and Beyond Burger.

[1] AAA, aromatic amino acid; SAA, sulfur amino acid; SE, standard error.

Table 4.10 (cont.)

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[2] DIAA reference ratios and DIAAS were calculated using the recommended indispensable amino acids scoring pattern, expressed as mg amino acid/g protein. Child: His 20, Ile 32, Leu 66, Lys 57, SAA 27, AAA 52, Thr 31, Trp 8.5, and Val 43 (FAO, 2013). Older child, adolescent and adult: His 16, Ile 30, Leu 61, Lys 48, SAA 23, AAA 41, Thr 25, Trp 6.6, and Val 40 (FAO, 2013).

[3] First-limiting AA in parentheses.

Table 4.11. Digestible indispensable amino acids (DIAA) reference ratio and digestible indispensable amino acid score (DIAAS) in food ingredients as measured in growing pigs[1,2]

	80% lean beef burger + bun				Pork burger + bun				Impossible Burger + bun			
Item	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE
Child (6 months to 3 years)[3]												
DIAA reference ratio												
His	1.18	1.17	0.01	0.02	1.31	1.35	-0.04^{+}	0.02	1.03	1.05	-0.02	0.02
Ile	1.19	1.19	0.00	0.02	1.24	1.28	-0.03^{+}	0.01	1.27	1.29	-0.02	0.02
Leu	1.00	0.99	0.00	0.01	1.02	1.04	-0.02	0.01	0.99	1.01	-0.01	0.02
Lys	0.99	0.97	0.02	0.02	1.00	1.00	0.00	0.02	0.73	0.71	0.01	0.02
SAA	1.08	1.08	0.01	0.02	1.14	1.19	-0.05*	0.01	0.97	1.02	-0.04	0.03
AAA	1.31	1.30	0.02	0.02	1.36	1.38	-0.02	0.02	1.51	1.53	-0.02	0.03
Thr	1.01	1.00	0.01	0.03	1.06	1.12	-0.05^{+}	0.02	0.94	0.97	-0.03	0.04
Trp	1.26	1.17	0.09**	0.02	1.34	1.35	0.00	0.02	1.37	1.36	0.01	0.03
Val	0.98	0.98	0.00	0.02	1.00	1.03	-0.03^{+}	0.01	1.02	1.04	-0.02	0.02
DIAAS[4], %	98 (Val)	97 (Lys)	-0.17	1.86	100	100	-0.83	1.59	73 (Lys)	71 (Lys)	1.38	1.62
Older child, adolescent, adult[3]												
DIAA reference ratio												
His	1.47	1.46	0.01	0.03	1.63	1.68	-0.05^{+}	0.02	1.29	1.31	-0.02	0.03

Table 4.11 (cont.)

Ile	1.27	1.27	0.00	0.02	1.33	1.36	-0.03^{+}	0.02	1.36	1.38	-0.02	0.02
Leu	1.08	1.07	0.00	0.01	1.10	1.13	-0.02	0.01	1.07	1.09	-0.02	0.02
Lys	1.17	1.16	0.02	0.02	1.19	1.19	0.00	0.02	0.86	0.85	0.02	0.02
SAA	1.27	1.27	0.01	0.03	1.34	1.39	-0.05*	0.02	1.14	1.19	-0.05	0.08
AAA	1.66	1.65	0.02	0.02	1.72	1.75	-0.03	0.02	1.92	1.94	-0.02	0.03
Thr	1.26	1.24	0.01	0.04	1.32	1.38	-0.06^{+}	0.03	1.16	1.20	-0.04	0.04
Trp	1.63	1.51	0.12**	0.02	1.73	1.73	0.00	0.03	1.76	1.75	0.01	0.03
Val	1.05	1.05	0.00	0.02	1.07	1.11	-0.03^{+}	0.01	1.10	1.12	-0.02	0.02
DIAAS[4], %	105	105	0.18	1.91	107	111	-3.17^{+}	1.53	86 (Lys)	85 (Lys)	1.62	1.93

[1] AAA, aromatic amino acid; SAA, sulfur amino acid; SE, standard error.

[2] Means in a row differ if *Measured vs. predicted $P \leq 0.05$, **Measured vs. predicted $P \leq 0.01$, or tend to differ if $^{+}$Measured vs. predicted $0.05 < P \leq 0.10$. Values are means and pooled SEs, n = 6.

[3] DIAA reference ratios and DIAAS were calculated using the recommended indispensable amino acids scoring pattern, expressed as mg amino acid/g protein. Child: His 20, Ile 32, Leu 66, Lys 57, SAA 27, AAA 52, Thr 31, Trp 8.5, and Val 43 (FAO, 2013). Older child, adolescent and adult: His 16, Ile 30, Leu 61, Lys 48, SAA 23, AAA 41, Thr 25, Trp 6.6, and Val 40 (FAO, 2013).

[4] First-limiting AA in parentheses.

Literature Cited

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

Adeola, O., Xue, P. C., Cowieson, A. J., and Ajuwon, K. M. 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim. Feed Sci. Technol. 221:274-283. doi:10.1016/j.anifeedsci.2016.06.004

Almeida, F. N., Htoo, J. K., Thomson, J., and Stein, H. H. 2014. Effects of heat treatment on apparent and standardized ileal digestibility of amino acids in canola meal fed to growing pigs. Anim. Feed Sci. Technol. 187:44-52. doi:10.1016/j.anifeedsci.2013.09.009

Almeida, F. N., Petersen, G. I., and Stein, H. H. 2011. Digestibility of amino acids in corn, corn co-products and bakery meal fed to growing pigs. J. Anim. Sci. 89:4109-4115. doi:10.2527/jas.2011-4143

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

Bailey, H. M., and Stein, and H. H. 2020a. Raw and roasted pistachio nuts (Pistacia vera L.) are ‘good’sources of protein based on their digestible indispensable amino acid score as determined in pigs. J. Sci. Food. Agric. 100:3878-3885. doi:10.1002/jsfa.10429

Bailey, H. M., Mathai, J. K., Berg, E. P., and Stein, H. H. 2020b. Most meat products have digestible indispensable amino acid scores that are greater than 100, but processing may increase or reduce protein quality. Br. J. Nutr. 24:14-22. doi:10.1017/S0007114520000641

Bailey, H. M., Mathai, J. K., Berg, E. P., and Stein, H. H. 2020c. Pork products have digestible indispensable amino acid scores (DIAAS) that are greater than 100 when determined in pigs, but processing does not always increase DIAAS. J. Nutr. 150:475-482. doi:10.1093/jn/nxz284

Beardsworth, A. D., and Keil E. T. 1991. Vegetarianism, veganism, and meat avoidance: recent trends and findings. Br. J. Nutr. 93:19-24. doi:10.1108/00070709110135231

Casas, G. A., Almeida, J. S., and Stein, H. H. 2015. Amino acid digestibility in rice co-products fed to growing pigs. Anim. Feed Sci. Technol. 207:150-158. doi:10.1016/j.anifeedsci.2015.05.024

Cervantes-Pahm, S. K., Liu, Y., and Stein, H. H. 2014. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br. J. Nutr. 111:1663-1672. doi:10.1017/S0007114513004273

Daniel, C. R., Cross, A. J., Koebnick, C., and Sinha, R. 2011. Trends in meat consumption in the USA. Public Health Nutr. 14:575-583. doi:10.1017/S1368980010002077

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

Fanelli, N. S., Bailey, H. M., Guardiola, L. V., and Stein, H. H. 2021. Values for digestible indispensable amino acid score (DIAAS) determined in pigs are greater for milk than for breakfast cereals, but DIAAS values for individual ingredients are additive in combined meals. J. Nutr. 151:540-547. doi:10.1093/jn/nxaa398

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation. Rome.

FAO. 2014. Research approaches and methods for evaluating the protein quality of human foods. Report of an FAO expert working group. Rome.

FAOSTAT. 2018. Food Supply - Crops Primary Equivalent. http://www.fao.org/faostat/en/#data/CC (accessed Apr. 2021).

Han, F., Han, F., Wang, Y., Fan, L., Song, G., Chen, X., Jiang, P., Miao, H., and Han, Y. 2019. Digestible indispensable amino acid scores of nine cooked cereal grains. Br. J. Nutr. 121:30-41. doi:10.1017/S0007114518003033

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

Hodgkinson, S. M., Stein, H. H., de Vries, S., Hendriks, W. H., and Moughan, P. J. 2020. Determination of true ileal amino acid digestibility in the growing pig for calculation of digestible indispensable amino acid score (DIAAS). J. Nutr. 150:2621-2623. doi:10.1093/jn/nxaa210

Jaeger, H., Janositz, A., and Knorr, D. 2010. The Maillard reaction and its control during food processing. The potential of emerging technologies. Pathol. Biol. 58:207-213. doi:10.1016/j.patbio.2009.09.016

Kim, B. G., Lee, S. A., Park, K. R., and Stein, H. H. 2020. At least three days of adaptation are required before indigestible markers (chromium, titanium, and acid insoluble ash) are stabilized in the ileal digesta of 60-kg pigs, but values for amino acid digestibility are affected by the marker. J. Anim. Sci. 98:1-8. doi:10.1093/jas/skaa027

Mathai, J. K., Liu, Y., and Stein, H. H. 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

Myers, W. D., Ludden, P. A., Nayigihugu, V., and Hess, B. W. 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

Ruby, M. B. 2012. Vegetarianism. A blossoming field of study. Appetite 58:141-150. doi:10.1016/j.appet.2011.09.019

Rutherfurd, S. M., Fanning, A. C., Miller, B. J., and Moughan, P. J. 2014. 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

Speedy, A. W. 2003. Global production and consumption of animal source foods. J. Nutr. 133:4048S-4053S. doi:10.1093/jn/133.11.4048S

Stein, H. H., Pedersen, C., Wirt, A. R., and Bohlke, R. A. 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., Seve, B., Fuller, M. F., Moughan, P. J., and de Lange, C. F. M. 2007. 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

Stein, H. H., Shipley, C. F., and Easter, R. A. 1998. Technical note: a technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433-1436. doi:10.2527/1998.7651433x

Stein, H. H., Trottier, N. L., Bellaver, C., and Easter, R. A. 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

US Department of Agriculture, Agricultural Research Service. 2019. USDA Food Composition Database. https://ndb.nal.usda.gov (accessed Dec 2020).

US Department of Agriculture, Agricultural Research Service. 2020. USDA Food Composition Database. https://ndb.nal.usda.gov (accessed Dec 2020).

United States Department of Agriculture and Food Safety and Inspection Service. 2016. Meat preparation. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation (accessed Aug. 2019)

U.S. Environmental Protection Agency. 2000. Acid digestion of sediments, sludges, and soils, U.S. EPA, Washington, DC.

Witte, B., Obloj, P., Koktenturk, S., Morach, B., Brigl, M., Rogg, J., Schulze, U., Walker, D., Von, K. E., Dehnert, N., and Grosse-Holz, F. 2021. Food for thought: The protein transformation. Boston Consulting Group. https://www.bcg.com/publications/2021/the-benefits-of-plant-based-meats (accessed Apr 2021).

Xue, P. C., Ragland, D., and Adeola, O. 2014. Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. J. Anim. Sci. 92:3937-3944. doi:10.2527/jas.2014-7815

CHAPTER 5: CONCLUSION

Amino acids (AA) are essential elements of a well-balanced eating plan and play a significant role in numerous biological functions. The excellence of a protein is determined by the amount of indispensable AA and how well it can be digested. Animal-based proteins generally contain more digestible AA than plant-based proteins. Nevertheless, many individuals lack access to high-quality protein due to poverty or insufficient knowledge about proper nutrition. As a result, ensuring adequate AA intake, particularly among vulnerable populations, is critical for maintaining good health and preventing chronic disorders.

Various approaches have been developed to evaluate protein quality in human foods, the most current being the digestible indispensable amino acid score (DIAAS). Results of this research demonstrated that the low protein quality for cereal-based ingredients such as cornflakes, quick oats, and burger buns can be compensated for by using animal-based products such as milk and meat-based ingredients in a mixed meal. However, when two cereal-based ingredients were used in a mixed meal, lysine was present as the first limiting AA, demonstrating that animal-based proteins are more suitable for complementing low quality plant-based proteins. This is important because individuals consume meals consisting of different foods, and the concept of complementarity of proteins must be demonstrated to allow a dietary consumption of adequate indispensable AA to meet AA requirements.

The additivity approach utilized in DIAAS was tested and confirmed in this research, ensuring that an adequate AA meal was met when complementary proteins were used and that the protein quality of a mixed meal is additive when standardized ileal digestibility (SID) values are used. This is important because DIAAS in mixed meals can be calculated from individual ingredients, providing a more practical method of meeting AA requirements. Whereas more research is needed to understand the potential benefits and limitations of additivity in individual ingredient DIAAS, establishing a comprehensive database on AA digestibility in different foods can provide a more practical framework for formulating dietary recommendations for mixed meals. It is also important to stress that DIAAS related only to quality of individual ingredients or mixed meals and further studies are necessary to determine the optimal consumption of ingredients in a mixed meal that meets AA requirements for humans. Furthermore, it is hypothesized that the concept of DIAAS additivity can be applied to all types of food, but additional research to validate this hypothesis is needed.