DIGESTIBLE INDISPENSABLE AMINO ACID SCORES FOR MEAT PRODUCTS

BY

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THESIS

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ABSTRACT

Two experiments were conducted to determine the digestible indispensable amino acid scores (**DIAAS**) for pork and beef products using the pig as a model, and to test the hypothesis that various degrees of meat processing may increase the digestibility of amino acids (**AA**) and protein quality of meat as shown by an increase in DIAAS. In experiment 1, DIAAS values were determined for 9 pork products (i.e., raw belly, smoked bacon, smoked-cooked bacon, non-cured ham, alternatively cured ham, conventionally cured ham, medium loin, medium-well loin, and well-done loin). Ten female pigs (BW: 26.63 ± 1.62 kg) were surgically fitted with a T-cannula in the distal ileum and randomly allotted to a 10 × 10 Latin square design with 10 diets and ten 7-d periods; the initial 5 d were for adaptation to the diet and the following 2 d for 9 h of ileal digesta collection. Nine diets contained a single pork product as the sole source of crude protein (**CP**) and AA. A N-free diet was formulated to determine basal endogenous losses of CP and AA, enabling the calculation of standardized ileal digestibility (**SID**) of AA. The DIAAS values were calculated using the determined concentration of digestible indispensable AA (**IAA**) in each meat product and 2 reference protein patterns established by the Food and Agriculture Organization of the United Nations (**FAO**); 1) children 6 mo to 3 yr and 2) children > 3 yr, adolescents, and adults. All pork products had a DIAAS value greater than 100, regardless of the reference protein pattern and processing method. When compared with the 2 human AA requirement patterns, Val was the limiting AA in all pork products, except for smoked-cooked bacon, which was limiting in Trp for children 6 mo to 3 yr. Medium loin had the greatest (P < 0.05) DIAAS value for children 6 mo to 3 yr, and smoked-cooked bacon had the greatest (P < 0.05) DIAAS value for older children, adolescents, and adults. Among the pork bellies, smoked-cooked bacon had the greatest (P < 0.05) DIAAS value with no difference observed between raw

belly and smoked bacon. The digestibility of IAA in smoked-cooked bacon was lower ($P <$ 0.05) than for raw belly, but no differences were observed between the other bellies. Among the pork hams, alternatively cured ham had the greatest ($P < 0.05$) value for DIAAS with no difference observed between conventionally cured and non-cured ham. Alternatively cured and conventionally cured ham had greater ($P < 0.05$) digestibility of IAA compared with non-cured ham. The loin cooked to the medium degree of doneness had a greater ($P < 0.05$) DIAAS value than the loins cooked to medium-well and well-done degrees of doneness, with no differences observed between the DIAAS of medium-well and well-done loin, as well as no differences were observed in the digestibility of IAA among all loins. Results indicate that pork products are excellent quality protein sources and that processing may increase DIAAS. Experiment 2 was conducted to determine the DIAAS values for 8 meat products (i.e., salami, bologna, beef jerky, raw ground beef, cooked ground beef, medium-rare ribeye roast, medium ribeye roast, and well-done ribeye roast). Nine ileal-cannulated female pigs (BW: 35.50 ± 3.77 kg) were randomly allotted to a 9 × 8 Youden square design with 9 diets and eight 7-d periods with ileal digesta collected for 9 h on d 6 and 7. Each of the 8 meat products were included in one diet as the sole source of CP and AA, and a N-free diet was formulated to determine basal endogenous losses of CP and AA. The SID of AA was calculated, and the concentration of digestible indispensable AA in each meat product was determined and compared with the 2 established reference protein patterns used in Exp. 1. The DIAAS was determined based on the limiting AA in the meat compared with the human AA requirements. For children 6 mo to 3 yr, sulfur AA were limiting in salami and beef jerky, Leu was limiting in bologna, cooked ground beef, and well-done ribeye roast, Trp was limiting in raw ground beef, and Val was limiting in medium-rare and medium ribeye roasts. Well-done ribeye roast and cooked ground beef had DIAAS values less than 100,

but all other meat products had values greater than 100. Medium ribeye roast and bologna had the greatest ($P < 0.05$) DIAAS values followed by raw ground beef, salami, medium-rare ribeye roast, beef jerky, well-done ribeye roast, and cooked ground beef, respectively. For older children, adolescents and adults, sulfur AA were limiting in beef jerky, Leu was limiting in bologna, raw ground beef, and cooked ground beef, and Val was limiting in salami and the 3 ribeye roasts. All meat products had DIAAS values greater than 100, except cooked ground beef with a DIAAS of 99. Medium ribeye roast and bologna had the greatest ($P < 0.05$) DIAAS values followed by raw ground beef, salami, beef jerky, medium-rare ribeye roasts, well-done ribeye roasts, and cooked ground beef, respectively. The digestibility of most IAA was not different among salami, bologna, beef jerky, and cooked ground beef, but the digestibility of IAA in these products was less ($P < 0.05$) than in raw ground beef. The digestibility of IAA in medium-rare ribeye roast was not different from raw ground beef and well-done ribeye roast, but greater ($P < 0.05$) than in medium ribeye roast. Results from this experiment indicate that meat products generally provide high quality protein, however, overcooking may reduce IAA digestibility and DIAAS. In conclusion, curing and moderate cooking may increase DIAAS, whereas grinding meat prior to some processing methods or overcooking may reduce the digestibility of IAA and DIAAS of meat products.

Key words: digestible indispensable amino acid scores, beef, pork, amino acid digestibility, protein quality

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CHAPTER 1: INTRODUCTION

The new methodology for evaluating the protein quality of human food proteins, digestible indispensable amino acid scores (**DIAAS**), was established following a Food and Agriculture Organization of the United Nations (**FAO**) Expert Consultation in 2011 (FAO, 2013). The DIAAS methodology assesses protein and amino acid (**AA**) concentration, bioavailability, and digestibility of a food item to determine the overall protein quality (FAO, 2013). The growing rat was the recommended animal model when using the previous method of protein quality evaluation, protein digestibility-corrected amino acid score (FAO, 1991). However, the FAO has since recognized that the growing pig is a more appropriate animal model for determining protein quality of human foods (FAO, 2013). Since the establishment of the DIAAS procedure, protein quality of cereal grains (Cervantes-Pahm et al., 2014; Mathai et al., 2017; Abelilla et al., 2018), plant proteins (Mathai et al., 2018), dairy proteins (Mathai et al., 2018), and meat proteins (Bindari et al., 2018; Hodgkinson et al., 2018) has been reassessed by determining DIAAS in pigs.

Data for DIAAS of commonly consumed meat products, such as pork and beef, are limited. The DIAAS for beef that had undergone 4 common methods of processing was reported and it was observed that beef has a DIAAS value of less than 100 (Hodgkinson et al., 2018). To our knowledge, DIAAS has not been determined in pork, which is the most widely consumed meat globally and beef being the third most widely consumed meat (FAO, 2014). Meat is a concentrated source of protein and contain adequate amounts of all indispensable AA to meet or exceed human requirements (Bender, 1992; WHO, 2007). However, meat, especially pork and beef, undergo some degree of processing prior to human consumption. Thermal processing leads to protein denaturation and protein aggregation (Yu et al., 2017), which may result in an increase in AA digestibility or a decrease in the bioavailability of AA depending on the temperature and time that is used in the process (Moughan, 2003; Yu et al., 2017). However, data demonstrating effects of different meat processing methods, such as grinding, cooking, curing, fermenting, or dehydrating, on the digestibility of AA as determined in animal models is limited.

The objectives of this research were to test the hypothesis that pork and beef are excellent quality proteins with DIAAS values greater than 100, and to determine the effect of various degrees of meat processing on digestibility of AA and DIAAS as determined in the pig.

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CHAPTER 2: DIGESTIBLE INDISPENSABLE AMINO ACID SCORES FOR MEAT PRODUCTS: REVIEW OF LITERATURE

INTRODUCTION: GLOBAL AND U.S. PRODUCTION OF MEAT

The 2016 global production of meat was estimated at 330 million metric tons (FAOSTAT, 2016). The United States (**U.S.**) produced approximately 23 million metric tons of red meat (i.e., beef, veal, pork, lamb, and mutton), which increased by 3% from 2016 to 2017 (USDA-ERS, 2018). The annual global production of pork and beef was approximately 118 million and 66 million metric tons, respectively (FAOSTAT, 2016). Asia, specifically China, and the European Union are the main producers of pork with an annual production of approximately 54 million and 24 million metric tons, respectively (FAOSTAT, 2016). North America, specifically the U.S., is the third greatest producer of pork with an annual production of approximately 11 million metric tons (FAOSTAT, 2016). The states in the U.S. that produce the greatest amount of pigs on a live weight basis are Iowa, North Carolina, Minnesota, Illinois, and Indiana (USDA-ERS, 2018). The top global producers of beef are the U.S. and Brazil with an annual production of approximately 11 million and 9 million metric tons, respectively; China produces less beef than the U.S. and Brazil with an annual production of approximately 7 million metric tons (FAOSTAT, 2016). The states in the U.S. with the greatest amount of cattle on feed are Nebraska, Texas, Kansas, Iowa, and Colorado (USDA-ERS, 2018).

The global annual per capita consumption of meat was estimated at 43.2 kg, with the annual consumption of pork and beef approximately 16.0 and 9.3 kg per capita, respectively (FAOSTAT, 2013). According to the United States Department of Agriculture (**USDA**), pork accounts for approximately 40.4% of global meat consumption and pork was consumed in a greater amount compared with chicken, beef, and mutton and goat, which were consumed in an amount of approximately 32.4%, 21.8%, and 5.3% of global consumption, respectively (USDA, 2018a). Per capita meat consumption varies based on region and is greatly influenced by wealth, for example the U.S., a developed country, consumes approximately 115 kg per capita of meat annually, whereas Nigeria, a developing country in Sub-Saharan Africa, has an annual per capita consumption of meat of approximately 9.2 kg (FAOSTAT, 2013).

The world population is growing at a rate of about 1% per year and by 2025 the global population is estimated to reach 8.1 billion people, with 95% of the growth occurring in developing countries (OECD-FAO, 2014). Global meat consumption is expected to grow at a rate of 1.7% per year with the majority of growth being concentrated in Asia, Latin America, and the Middle East (Henchion et al., 2014), and by 2050 the demand for animal proteins in developing countries is predicted to double (Smith et al., 2018). The high protein quality in meat in combination with the increase in meat consumption in developing countries may assist the Food and Agriculture Organization of the United Nations (**FAO**) in reaching their goal of ending hunger and preventing malnutrition by 2030 (Bender, 1992; FAO et al., 2017).

PROTEIN AND AA COMPOSITION OF BEEF AND PORK

'Red meat' refers to meat from the postmortem muscle of mammalian species, e.g., cattle, pigs, goats, sheep, and deer (Bender, 1992). Red meat provides a variety of micronutrients, many of which are difficult to consume in adequate amounts from plant or cereal based foods, such as Zn, Fe, I, vitamin A, B12, and B6 (Bender, 1992). The crude protein (**CP**) and amino acid (**AA**) concentration in the lean portion of the carcass (the muscle) is generally similar in different cuts of muscle and constant among species with CP of approximately 19 to

22 g per 100 g of fresh meat (Bender, 1992; Franco et al., 2010). Ground meat, comprised of lean muscle and no greater than 30% fat (USDA-FSIS, 2005), has a constant CP and AA concentration among species with all indispensable AA (**IAA**) increasing after cooking (Table 2.1). Commercial cuts of red meat may differ in IAA concentration due to the type of muscle fibers present (Franco et al., 2010; Wu et al., 2016), for example connective tissue is comprised of collagen and elastin, which are limiting in sulfur AA (**SAA**) and Trp (Bender, 1992). The minor differences in IAA concentrations may be a result, in part, of method of analysis or moisture and fat concentration in the meat product (Beach et al., 1943; Bender, 1992; Rutherfurd and Moughan, 2000). Across all species and independent of cooking, Lys is the IAA in greatest concentration followed by Leu and Arg, respectively. The IAA in least concentration is Trp followed by Met as the second lowest concentrated IAA in red meat. Nonetheless, Table 2.2 shows that cooked beef and pork have greater amounts of IAA compared with the requirements for adult humans determined by the World Health Organization (**WHO**), except for Trp in cooked beef. Based on composition, meat products are excellent sources of protein and are able to meet or exceed the adult requirement for AA.

EFFECT OF MEAT PROCESSING ON PROTEIN QUALITY

The FAO has defined processed food as any food item that has been deliberately altered from its natural state affecting its eating quality or shelf life (FAO, 2004). This definition includes minimal processing, such as grinding, mincing, freezing, or packaging; and further processing, such as thermal processing, dehydrating, curing, fermenting, or the addition of approved ingredients (Seman et al., 2018). Meat undergoes some degree of processing prior to human consumption (Seman et al., 2018), with a variety of techniques implemented to meet consumer demand for improved flavor (Prestat et al., 2002), color (García-Segovia et al., 2007), and shelf-life (Gould, 1996). Meat processing is also essential for microbial control and food safety (Nummer et al., 2004). The digestibility of AA may be increased post-processing (Yu et al., 2017), however, AA bioavailability may decrease due to the structural transformation of AA during some processing methods (Moughan, 2003).

Cooking, or thermal processing, meat products is essential in the destruction of microorganisms and in the improvement of sensory characteristics (Gould, 1996; Prestat et al., 2002). The USDA has determined safe minimum internal temperatures that are recommended to eliminate pathogenic organisms in meat products (USDA, 2018b). Ground meat and whole cuts of red meat (i.e., steaks, chops, or roasts from beef, pork, veal, or lamb) are recommended to be cooked to an internal temperature of 160 ºF (71.1 ºC) and 145 ºF (62.8 ºC) with an additional rest period of 3 minutes, respectively (USDA, 2018b).

Protein goes through conformational changes at different temperatures during thermal processing (Tornberg, 2015; Yu et al., 2017). The initial stage of protein unfolding, involving the tertiary and secondary structures, is referred to as protein denaturation (Yu et al., 2017). Denaturation of the secondary structure results in protein-protein interactions and protein aggregation occurs (Tornberg, 2005; Yu et al., 2017). Protein denaturation in meat is observed at temperatures of approximately 70 ºC (Di Luccia et al., 2015), however, this may vary based on the muscle fiber type (Yu et al., 2014). Denaturation may increase digestibility of AA (Bax et al., 2012; Li et al., 2017), whereas, protein aggregation is observed at greater temperatures, such as 100 ºC or greater, and negatively affects the bioavailability of AA in addition to decreasing AA digestibility (Gatellier and Santé-Lhoutellier, 2009; Gatellier et al., 2010).

Individual AA, such as basic AA (i.e., Arg, Lys, His), aromatic AA (**AAA**; i.e., Phe, Trp, Tyr), and cysteine, are prone to reactions with free radicals during processing (Gatellier et al., 2009). Lysine is particularly unstable during thermal processing, however, when pork was cooked at 60 ºC, approximately 90% of Lys was retained in the meat and no significant decrease in the concentration of Lys was observed when temperatures reached 75 ºC (Wilkinson et al., 2014). Gatellier et al. (2009) observed no effect on the concentration of AAA when beef was cooked at 60 ºC, whereas after 30 min of cooking at 100 and 140 ºC, the concentration of Phe, Trp, and Tyr was reduced by 38 and 78%, 32 and 46%, and 69 and 93%, respectively (Gatellier et al., 2009). Similarly, Gatellier et al. (2010) observed a decrease in the concentration of AAA after beef was cooked to 207 ºC, with Tyr being the least stable AAA. In pork, a 50% reduction in the concentration of Trp was observed after cooking for 20 min at 102 ºC (Hernández-López et al., 2016). Wilkinson et al. (2014) analyzed the concentration of protein and AA in pork after cooking at 60 and 75 ºC, and observed approximately 89 and 82% of protein remaining in the meat, respectively. The percentage of individual AA retained in the meat after cooking was similar to that of protein with the exception of His and taurine, which were the least stable AA with concentrations decreasing approximately 17 and 25% at 60 ºC and 30 and 48% at 75 ºC, respectively (Wilkinson et al., 2014).

The bioavailability of AA is only one aspect that determines the protein quality of a food item; protein quality also takes into account the digestibility of AA, which may be affected by processing (Bax et al., 2013; Hodgkinson et al., 2018). Hodgkinson et al. (2018) conducted an AA digestibility trial using the pig as a model and observed minimal effect of cooking beef to 71 ºC, except the digestibility of His was significantly lower in pan-fried beef compared with raw beef. However, pan-fried and boiled beef had greater concentrations of digestible AA compared with raw beef. The pan-fried beef was exposed to a surface temperature of 186 ºC for a short period of time, approximately 12 min, and the boiled beef was cooked for approximately 15 min but was exposed to a lower surface temperature of 80 ºC (Hodgkinson et al., 2018). The combination of cooking time and temperature for the two beef products may have resulted in moderate protein denaturation explaining the increase of digestible AA. Similarly, an animal trial conducted by Bax et al. (2013) observed no effect on the digestibility of individual AA in beef cooked at temperatures of 60, 75, and 95 ºC for 30 min. Rutherfurd et al. (2014) conducted a digestibility trial in rats and observed a 30 and 58% increase in AA digestibility of poor quality proteins, zein and blood meal, respectively, after undergoing moderate oxidation. However, the mean digestibility of AA in beef muscle protein decreased approximately 15% after oxidation (Rutherfurd et al., 2014).

The digestibility of AA may be affected by additional processing methods, such as grinding (Rémond et al., 2007; Pennings et al., 2013; Soladoye et al., 2015). Grinding prior to consumption, similar to chewing, reduces the particle size of a food item and increases the surface area, therefore, improving the efficiency of proteolytic enzymes and resulting in an increase in AA digestibility (Soladoye et al., 2015; Pennings et al., 2013). Postprandial increase in plasma Phe was greater after human subjects consumed minced beef compared with beef steak (Pennings et al., 2013). In addition, plasma Leu concentration was greater in human subjects with natural dentition than full-denture wearers, representing different chewing efficiencies, after consuming sliced beef (Rémond et al., 2007). In contrast, grinding prior to thermal processing increases the surface area resulting in greater exposure of protein to oxygen, potentially leading to decreased AA digestibility (Rutherfurd et al., 2014; Soladoye et al., 2015).

Certain approved ingredients, such as salt, nitrite, nitrate, and reducing agents (i.e., ascorbates and erythorbates), may provide oxidative stability to meat during processing, protecting against loss of IAA and protein aggregation (Lund et al., 2011; Van Hecke et al., 2014; Soladoye et al., 2015). Addition of sodium/potassium nitrite or nitrate and salt is associated with curing, approximately 70% of pork is cured with nitrite (Ramarathnam and Rubin, 1994). These ingredients are added for a variety of reasons, such as to extend product shelf-life, improve food safety, slow rancidity, and enhance sensory characteristics (Ramarathnam and Rubin, 1994). Nitrites and nitrates are primarily added to produce the characteristic red color of cured meat and to inhibit the growth of microorganisms, specifically suppressing *Clostridium botulinum* (Seman et al., 2018). The combination of nitrite and reducing agents (i.e., ascorbic acid) may protect against or partially inhibit protein oxidation in meat (Honikel, 2008). In dry cured meat, water activity is decreased and the concentration of free AA has been observed to increase (Toldrá and Aristoy, 1993). However, limited data are available on the bioavailability and digestibility of AA in cured and dehydrated meat products.

TRANSITION FROM PDCAAS TO DIAAS

In 1989, the Protein Digestibility-Corrected Amino Acid Score (**PDCAAS**) was recommended as the appropriate method for evaluating protein quality during the Joint Food and Agriculture Organization of the United Nations (**FAO**) and World Health Organization (**WHO**) Expert Consultation on Protein Quality Evaluation (FAO, 1991). In 2011, after 20 yr of application of the PDCAAS method, an FAO Expert Consultation was held to review its adequacy and limitations (FAO, 2013). At the conclusion of the consultation, the Digestible Indispensable Amino Acid Score (**DIAAS**) was recommended to replace PDCAAS as the appropriate method for dietary protein quality assessment. However, prior to the use of DIAAS in practice a dataset with values for ileal digestibility of AA in a sufficient number of human foods determined in both human and animal models is required (FAO, 2013).

PDCAAS

The PDCAAS method relies on determination of protein content, AA concentration, and protein digestibility in a food item (Boye et al., 2012). Protein quality as determined by PDCAAS is expressed as the AA in lowest concentration (the limiting AA) in the test protein divided by the same AA in a reference protein for a specific age group, and then corrected for the protein digestibility of the test protein (FAO, 1991). Calculation of PDCAAS is accomplished using the following formula (FAO, 1991):

PDCAAS = [(mg of limiting AA in 1 g test protein / mg of same AA in reference protein) × fecal digestibility of protein].

A number of limitations relating to PDCAAS have been documented (WHO, 2007; Boye et al., 2012; Gilani, 2012; Schaafsma, 2012; FAO, 2013). First, the ideal reference protein pattern recommended for all age groups, except infants, is based on AA requirements for preschool children 2 to 5 yr calculated by dividing the AA requirements by the safe level (upper range) of protein intake (FAO, 1991). Amino acid requirements vary with physiological state and health status (WHO, 2007). Therefore, protein needs may be underestimated and the protein quality may be overestimated in situations where specific AA requirements may be increased, such as in pregnancy, old age, or in individuals with negative energy balance.

Total tract digestibility of protein determined in rats is required and the assumption that all AA have the same digestibility value as protein is made (FAO, 1991). However, bioavailability of AA differ from total protein (Darragh and Hodgkinson, 2000; Moughan, 2003;

Gilani et al., 2012a), and ileal digestibility values are more accurate than total tract digestibility for AA especially when antinutritional factors are present in ingredients (Rowan et al., 1994; Gaudichon et al., 2002; Moughan, 2003; Gilani et al., 2012b). In addition, the pig is a more appropriate model than the rat for determining ileal digestibility of AA for humans (Rowan et al., 1994; Deglaire et al., 2009; Deglaire and Moughan, 2012).

Values for PDCAAS are also truncated to 1.0, or 100%, which may underestimate the value of high quality and complementary proteins (FAO, 1991). For example, 2 proteins such as milk and soy protein have reported PDCAAS values of 100 (Boye et al., 2012), but milk protein has calculated values for AA ratios (digestible AA profile of the food item divided by AA pattern of the reference protein) greater than soy protein (Rutherfurd et al., 2015). Consequently, milk protein would have a greater ability in complementing lower quality proteins and add greater value to diets inadequate in IAA than soy protein. This is an important limitation because food items are almost always consumed in a mixture.

DIAAS

The DIAAS methodology estimates protein quality by measuring the digestibility of each AA in a food item at the end of the small intestine (the ileum) of humans, the growing pig, or the growing rat. The concentration of digestible IAA in 1 g protein of the food item is calculated and compared with one of 3 reference protein patterns. The DIAAS value for the test protein is the least digestible IAA when compared to an AA reference pattern. The following formula is used to calculate DIAAS (FAO, 2013):

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

A major difference between PDCAAS and DIAAS is that it is recognized that when digestibility values cannot be determined in the human, the growing pig is the most appropriate animal model (FAO, 2013), which is supported by the literature (Rowan et al., 1994; Moughan, 2003; Deglaire et al., 2009). In addition, the ileal digestibility of each IAA, rather than total tract digestibility of protein, is determined in the test protein and corrected for basal endogenous losses of AA measured at the terminal ileum.

For regulatory purposes, DIAAS enables protein quality claims to be made based on the protein concentration of a food item as well as the quality and bioavailability of AA. A food item can be considered a 'good' or 'excellent' quality protein if the DIAAS value is between 75 and 100 or greater than 100, respectively, and no protein claim can be made for food items with a DIAAS value below 75 (FAO, 2013). Protein claims for DIAAS indicate that protein quality cannot be substituted for quantity. As an example, legumes contain an equal or greater amount of protein per 100 g compared with some animal proteins, but because of the poor AA composition, legumes have DIAAS values less than 75 and, therefore, no protein claim can be made for legumes (Shaheen et al., 2016; USDA, 2018a).

Protein claims can be assigned to a food item for 3 different age groups under the DIAAS method. In contrast to PDCAAS, 3 reference protein patterns have been established for DIAAS; 1) infants birth to 6 mo, 2) young children 6 mo to 3 yr, and 3) children older than 3 yr, adolescents, and adults (FAO, 2013). The scoring patterns are derived from a 2007 report on protein and AA requirements in human nutrition and computed as the AA requirements divided by the mean protein requirement for a specific age group, except for infants which has a pattern based on the AA composition of human breast milk (WHO, 2007). The 2011 FAO Expert Consultation recognizes the need for further research on human AA requirements in different health circumstances, such as malnutrition, pregnancy, lactation, aging, and athletes to accurately determine protein quality in those groups (FAO, 2013).

The DIAAS methodology also recognizes higher quality proteins by eliminating the truncation of scores that exceed 100%. Elimination of truncation enables protein values of mixed meals to be calculated. For example, milk has a DIAAS greater than 100, which can complement wheat that has a DIAAS of approximately 50 (Mathai et al., 2017). In addition, by analyzing the digestibility of each AA, complementary proteins can be documented, for example rice, a cereal grain, has a low concentration of digestible Lys, but a high concentration of digestible SAA, and therefore, can complement peas, a plant protein, that is low in digestible SAA and high in digestible Lys (Cervantes-Pahm et al., 2014; Mathai et al., 2017). Food proteins are normally consumed in combination with other ingredients as part of a meal. Therefore, prior to the implementation of DIAAS for regulatory purposes and its use in assessing the protein quality of complete meals, ileal digestibility values for IAA across foods are needed.

DIAAS OF FOOD PROTEINS

A report from the sub-committee of the 2011 FAO Consultation on protein quality, compiled published ileal AA digestibility data for human food proteins (Moughan et al., 2011). The compiled data represent ileal AA digestibility as determined in the human, growing pig, and growing rat from a large number of studies published over the years. Thus, a variety of methodologies were used resulting in inherent variation. Therefore, there is a need to form a comprehensive standardized dataset with current methodologies for obtaining ileal AA digestibility data in the growing pig.

Several cereal grains have recently been assigned DIAAS values as determined in the growing pig (Cervantes-Pahm et al., 2014; Mathai et al., 2017; Abelilla et al., 2018), and the growing rat (Rutherfurd et al., 2015). The DIAAS values for cereal grains are shown in Table 2.3. Oats had a DIAAS of 77 and can be considered a 'good' quality protein for older children, adolescents, and adults (Cervantes-Pahm et al., 2014); however, when oats were cooked, the DIAAS value is reduced and is less than the minimum score required for a protein claim (Rutherfurd et al., 2015). Cereal grains contribute approximately 54% of calories in developing countries (Kearney, 2010), but the majority of cereal grains have a DIAAS value below 75 and an inadequate amount of IAA, especially Lys, compared with specific age group AA requirements (Cervantes-Pahm et al., 2014; Rutherfurd et al., 2015; Mathai et al., 2017; Abelilla et al., 2018). In contrast, plant proteins, such as pea protein, soy isolate, soy flour, and cooked beans, had DIAAS values greater than cereal grains and were consistently limiting in SAA (Rutherfurd et al., 2015; Mathai et al., 2017). The majority of the legume proteins can be considered 'good' quality proteins and soy flour can be claimed as an 'excellent' quality protein (Table 2.4). Several animal proteins, i.e., dairy and meat (Tables 2.5 and 2.6) have been evaluated for protein quality utilizing the DIAAS method (Rutherfurd et al., 2015; Mathai et al., 2017; Bindari et al., 2018; Hodgkinson et al., 2018). Dairy proteins, such as whey isolate, whey concentrate, milk concentrate, and skimmed milk powder, are all considered 'excellent' quality proteins as determined in the pig (Mathai et al., 2017). The SAA were in least concentration for the milk proteins compared with the age specific AA requirement, whereas His was limiting in the whey proteins (Rutherfurd et al., 2015; Mathai et al., 2017). Meat proteins, such as pork and beef tissue hydolyzates, varied greatly in protein quality and limiting AA (Bindari et al., 2018), whereas whole beef steaks were 'good' quality proteins that increased with certain processing techniques, i.e., boiling and pan-frying, and were consistently limiting in Val (Hodgkinson et al., 2018). A variety of human food proteins have been analyzed using the DIAAS methodology, however, DIAAS data are lacking for mixed diets. Rutherfurd et al. (2015) calculated the DIAAS of a mixed diet composed of 60% milk and 40% breakfast cereal. The calculation demonstrated that the protein quality of a commonly consumed breakfast cereal (DIAAS of 1) in developed countries may be greatly improved when mixed with a milk protein (DIAAS of 118).

CONCLUSIONS

Meat is consumed in various amounts throughout the world and is a concentrated source of protein and AA with limited variations in the raw product among species. The concentration of IAA in meat are generally greater than or equal to the human AA requirements. However, humans rarely eat meat without applying some degree of processing, especially when consuming pork and beef products; and processing methods may have an effect on the concentration and bioavailability of AA in meat products.

The new methodology for evaluating protein quality in human food proteins, DIAAS, assesses the concentration of AA as well as the digestibility of AA in a food item. However, data are limited on how the digestibility of AA change after various degrees of processing are applied to a meat product. Consequently, there is a need to determine the digestibility of AA in meat products using the pig as a model for humans to adequately evaluate protein quality in these products. Thus, it is our hypothesis that meat is an excellent quality protein, as determined in pigs using the DIAAS methodology, and that the protein quality of meat may improve with various processing techniques.

TABLES

Table 2.1. Concentration of crude protein (CP) and amino acids (AA) in cooked and raw red meat (g per 100g edible portion)[1,2,3]

	CP	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Beef											
Raw	17.17	1.12	0.56	0.76	1.34	1.42	0.44	0.67	0.67	0.09	0.84
Cooked	27.73	1.80	0.90	1.23	2.16	2.30	0.71	1.08	1.07	0.14	1.36
Pork											
Raw	16.88	1.05	0.67	0.79	1.35	1.52	0.45	0.67	0.77	0.21	0.92
Cooked	25.69	1.60	1.03	1.20	2.06	2.31	0.68	1.03	1.17	0.33	1.39
Lamb											
Raw	16.56	0.98	0.52	0.80	1.29	1.46	0.43	0.67	0.71	0.19	0.89
Cooked	24.75	1.47	0.78	1.19	1.93	2.19	0.64	1.01	1.06	0.29	1.34
Veal											
Raw	18.58	1.09	0.67	0.92	1.48	1.53	0.43	0.75	0.81	0.19	1.03
Cooked	24.38	1.43	0.89	1.20	1.94	2.01	0.57	0.98	1.07	0.25	1.35
Deer											

Table 2.1 (cont.)

Raw	21.78	1.29	0.65	0.93	1.65	1.76	0.51	0.82	0.82	0.19	1.05
Cooked	26.45	1.58	0.79	1.13	2.01	2.14	0.62	1.00	1.00	0.23	1.28
Bison											
Raw	18.67	1.27	0.68	0.90	1.60	1.73	0.51	0.79	0.85	0.14	1.00
Cooked	23.77	1.62	0.87	1.15	2.04	2.21	0.64	1.01	1.08	0.18	1.28

[1]Values reported by USDA, 2018a.

[2]Raw meat was ground.

[3]Cooked meat was ground and broiled, except the cooking method for pork was unknown.

Table 2.2. Percentage of adult indispensable amino acid (IAA) requirements provided by 100 g of cooked beef or pork.[1,2]

	Adult IAA requirement (mg/g protein)[3]	Beef, % of requirement	Pork, % of requirement
His	15	216	266
Ile	30	147	156
Leu	59	132	136
Lys	45	184	200
SAA[4]	22	164	178
AAA[5]	38	183	197
Thr	23	168	199
Trp	6	85	211
Val	39	126	139

[1]Beef and pork amino acid (**AA**) composition reported by USDA, 2018a.

[2]Requirements reported by WHO, 2007 for persons greater than 18 yr old.

[3]Beef was ground and broiled prior to analysis and pork was ground and cooked, however, the cooking method was unknown.

[4]SAA = sulfur AA.

[5]AAA = aromatic AA.

Table 2.3. Digestible indispensable amino acid scores (DIAAS) for cereal grains reported in the literature[1,2,3]

Cereal grain	Animal model	Reference protein pattern		
		Infants (0 to 6 mo)	Young children (6 mo to 3 yr)	Older children, adolescents, and adults
Barely[4]	Pig	--	--	51 (Lys)
Corn[4]	Pig	--	--	48 (Lys)
Corn-based breakfast cereal[5]	Rat	--	1 (Lys)	--
Oats, raw[4]	Pig	--	--	77 (Lys)
Oats, cooked[5]	Rat	--	54 (Lys)	--
Oat protein concentrate[6]	Pig	41 (AAA)	56 (Lys)	67 (Lys)
Rice, raw[4]	Pig	--	--	64 (Lys)
Rice, cooked[5]	Rat	--	60 (Lys)	--
Rice protein concentrate[5]	Rat	--	37 (Lys)	--
Rye[4]	Pig	--	--	47 (Lys)
Sorghum[4]	Pig	--	--	29 (Lys)
Wheat[4,7]	Pig[4,7]	37 (Lys)[7]	54 (Lys)[7]	43 (Lys)[4]
Wheat bran[5]	Rat	--	41 (Lys)	--

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

Table 2.3 (cont.)

[2]Unreported DIAAS values for certain reference patterns are noted by "--".

[3]AAA = aromatic AA.

[4]Values reported by Cervantes-Pahm et al., 2014.

[5]Values reported by Rutherfurd et al., 2015.

[6]Values reported by Abelilla et al., 2018.

[7]Values reported by Mathai et al., 2017.

Table 2.4. Digestible indispensable amino acid scores (DIAAS) for plant proteins reported in the literature[1,2,3]

Plant protein	Animal model	Reference protein pattern		
		Infants (0 to 6 mo)	Young children (6 mo to 3 yr)	Older children, adolescents, and adults
Kidney beans, cooked[4]	Rat	--	59 (SAA)	--
Peas, cooked[4]	Rat	--	58 (SAA)	--
Pea protein concentrate[4,5]	Rat[4], Pig[5]	45 (Trp)[5]	82 (SAA)[4], 73 (SAA)[5]	--
Peanuts, roasted[4]	Rat	--	43 (Lys)	--
Soy flour[5]	Pig	73 (SAA)	105 (SAA)	--
Soy protein isolate[4,5]	Rat[4], Pig[5]	68 (SAA)[5]	90 (SAA)[4], 98 (SAA)[5]	--

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

[2]Unreported DIAAS values for certain reference patterns are noted by "--".

[3]SAA = sulfur AA.

[4]Values reported by Rutherfurd et al., 2015.

[5]Values reported by Mathai et al., 2017.

Table 2.5. Digestible indispensable amino acid scores (DIAAS) for dairy proteins reported in the literature[1,2,3]

Dairy protein	Animal model	Reference protein pattern		
		Infants (0 to 6 mo)	Young children (6 mo to 3 yr)	Older children, adolescents, and adults
Milk protein concentrate[4,5]	Pig[4], Rat[5]	85 (Trp)[4]	141 (SAA)[4], 118 (SAA)[5]	--
Skimmed milk powder[4]	Pig	81 (Thr)	123 (SAA)	--
Whey protein concentrate[4,5]	Pig[4], Rat[5]	71 (AAA)[4]	133 (His)[4], 97 (His)[5]	--
Whey protein isolate[4,5]	Pig[4], Rat[5]	67 (AAA)[4]	125 (His)[4], 109 (His)[5]	--

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

[2]Unreported DIAAS values for certain reference patterns are noted by "--".

[3]SAA = sulfur AA; AAA = aromatic AA.

[4]Values reported by Mathai et al., 2017.

[5]Values reported by Rutherfurd et al., 2015.

Table 2.6. Digestible indispensable amino acid scores (DIAAS) for meat reported in the literature[1,2,3]

Meat	Animal model	Reference protein pattern		
		Infants (0 to 6 mo)	Young children (6 mo to 3 yr)	Older children, adolescents, and adults
Beef, raw[4]	Pig	--	97 (Val)	--
Beef, boiled[4]	Pig	--	99 (Val)	--
Beef, grilled[4]	Pig	--	80 (Val)	--
Beef, pan-fried[4]	Pig	--	98 (Val)	--
Beef, roasted[4]	Pig	--	91 (Val)	--
Bovine collagen hydrolyzate[5]	Pig	-1 (Trp)	-2 (Trp)	-2 (Trp)
Bovine muscle hydrolyzate[5]	Pig	32 (Trp)	63 (Trp)	81 (Trp)
Porcine heart hydrolyzate[5]	Pig	38 (Trp)	76 (Trp)	87 (Ile)
Porcine muscle hydrolyzate[5]	Pig	21 (Trp)	42 (Trp)	54 (Trp)
Porcine plasma hydrolyzate[5]	Pig	60 (Ile)	87 (SAA)	102 (SAA)

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

[2]Unreported DIAAS values for certain reference patterns are noted by "--".

Table 2.6 (cont.)

[3]SAA = sulfur AA.

[4]Values reported by Hodgkinson et al., 2018.

[5]Values reported by Bindari et al., 2018.

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CHAPTER 3: PORK PRODUCTS HAVE DIGESTIBLE INDISPENSABLE AMINO ACID SCORES THAT ARE GREATER THAN 100, BUT PROCESSING DOES NOT ALWAYS INCREASE AMINO ACID SCORES

ABSTRACT

The objective was to determine digestible indispensable amino acid scores (**DIAAS**) for pork products and to test the hypothesis that processing may increase protein quality. Ten ileal cannulated female pigs (BW: 26.63 ± 1.62 kg) were randomly allotted to a 10 × 10 Latin square design with 10 diets and ten 7-d periods. Ileal digesta were collected from cannulas for 9 h on d 6 and 7. Nine diets contained a single pork product (i.e., raw belly, smoked bacon, smoked-cooked bacon, non-cured ham, alternatively cured ham, conventionally cured ham, medium loin, medium-well loin, and well-done loin) as the sole source of amino acids (**AA**). A N-free diet was formulated to determine basal endogenous losses of crude protein (**CP**) and AA enabling the calculation of standardized ileal digestibility (**SID**) of AA. The concentration of SID of AA in each pork product was calculated and compared with reference proteins established by the Food and Agriculture Organization of the United Nations to determine DIAAS. All pork products had DIAAS values greater than 100, regardless of processing, confirming the excellent quality of pork protein. The medium loin heated to 63 ºC had the greatest (P < 0.05) DIAAS for children 6 mo to 3 yr and the smoked-cooked bacon that was cured and fully cooked had the greatest (P < 0.05) DIAAS value for children older than 3 yr, adolescents, and adults. Raw belly, smoked bacon, and medium-well and well-done loins heated to 68 and 72 ºC, respectively, had the least (P < 0.05) DIAAS values for both scoring patterns. Alternatively cured ham that was cured with celery salt had greater (P < 0.05) DIAAS compared with the non-cured ham and the ham cured

more traditionally with sodium nitrite derived from Prague powder. In conclusion, pork is a high quality protein source and processing, i.e. curing and moderate heating, may increase DIAAS.

Key words: amino acids, digestible indispensable amino acid scores, protein quality, digestibility, pork, meat processing

INTRODUCTION

Pork is the most widely consumed animal meat protein in the world accounting for over 36% of the global meat intake (FAO, 2014). Pork is also a concentrated source of protein providing adequate amounts of all essential amino acids (**AA**) (Bender, 1992; USDA, 2018). In many countries, pork is the meat of choice, but consumption varies depending on the region with annual per capita consumption ranging from 2 kg in developing countries to 70 kg in some developed countries (FAOSTAT, 2013). Meat is highly perishable, therefore, processing almost always takes place prior to consumption to slow or inhibit microbial growth. Desirable sensory attributes are also developed with various forms of processing. Consequently, thermal processing induces modification to the 3-dimentional structure of the proteins, which may lead to increased digestibility of AA (Santé-Lhoutellier et al., 2008; Yu et al., 2017).

Protein quality is evaluated in human foods by using the Digestible Indispensable Amino Acid Score (**DIAAS**) described as the digestibility of individual dietary indispensable AA (**IAA**) compared with the same IAA in the reference protein (FAO, 2013). The digestibility of each IAA is determined at the end of the small intestine (the ileum) and corrected for basal endogenous losses. Human data are preferred, but if unavailable, the pig is recognized as an appropriate model for determining DIAAS for human foods (FAO, 2013).

The effect of cooking on protein modification has been studied (Santé-Lhoutellier et al., 2008; Di Luccia et al., 2015; He et al., 2018), and DIAAS has been determined in bovine meat cooked by various techniques and to a common internal temperature (Hodgkinson et al., 2018). Yet to our knowledge, there are no reported DIAAS values for pork products and the IAA digestibility of pork after processing has not been reported. Therefore, the hypothesis for the present work was that pork will have high DIAAS values and that processing may increase DIAAS.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment.

Preparation of Ingredients

Nine pork products were collected and prepared for DIAAS analysis at the Meat Science Laboratory at North Dakota State University, Fargo, ND (Tables 3.1 and 3.2). All raw or processed products were obtained from a single commercial source that is an international supplier of pork products. Processing procedures for all pork products are described in Table 3.1. Of the 9 products, there were 3 sources of belly including unprocessed raw belly, cured and smoked (partially cooked) bacon, and smoked-cooked bacon that was cured, sliced and fully cooked in a commercial continuous flow microwave baking oven. There were also 3 sources of fully cooked ham including non-cured ham, alternatively cured ham that was cured using celery salt, which contains naturally high levels of sodium nitrate, and conventionally cured ham that was cured with pink Prague powder, a mixture of sodium chloride and sodium nitrite. The 3 sources of pork loins were cooked to 3 designated endpoint temperatures, 63 (medium), 68 (medium-well), and 72 ºC (well-done), respectively. The raw, primal cuts can be identified in accordance to The Meat Buyers Guide (North American Meat Processors Association, 2007) and Institutional Meat Purchasing Specifications (**IMPS**) sections for ham (IMPS #402F), pork belly (IMPS #408), and loin (IMPS #413). The smoked bacon and smoked-cooked bacon were processed in accordance with Appendix A of the Food Safety and Inspection Service (**FSIS**) Compliance Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products (USDA-FSIS, 2017). The raw belly and all ham and loin ingredients were coarse ground, packaged, and frozen at North Dakota State University. The smoked bacon and smoked-cooked bacon were chopped in a 10-cup food processor (Black + Decker Inc., Towson, MD) before use at the University of Illinois. All pork products were vacuum packaged before being shipped to the University of Illinois, where they were stored at -20 ºC until use.

Animals, Housing, Diets, and Feeding

Ten growing, heterozygous Yorkshire female pigs (initial BW: 26.63 ± 1.62 kg) were surgically fitted with a T-cannula in the distal ileum as described by Stein et al. (1998). Pigs were housed in an environmentally controlled room in individual pens (2 × 3 m) equipped with smooth plastic siding, partially slatted floors, a feeder, and a nipple drinker. Following a 7-d recovery period from surgery, pigs were allotted to a 10 × 10 Latin square design with 10 diets and ten 7-d periods. Diets were randomly assigned in such a way that no pig received the same diet more than once during the experiment and there was, therefore, 10 replicate pigs per treatment.

A single pork product was included in 9 of the diets as the only crude protein (**CP**) and AA containing ingredient (Tables 3.3 and 3.4). A N-free diet was also formulated and fed to determine basal endogenous losses of CP and AA from the pigs, which was necessary for the calculation of DIAAS values. Titanium dioxide was included in the N-free diet at 0.5% as an indigestible marker. Vitamins and minerals were also included in the N-free diet to meet or exceed current nutrient requirements for growing pigs (NRC, 2012). The N-free diet was fed as-is, but all pork products were combined with sufficient quantities of the N-free diet to provide a diet containing approximately 16% CP on an as-fed basis.

Feed was provided daily in an amount equivalent to 4% of BW for each pig. Feed allowances were supplied in 2 equal meals at 0800 and 1700 h, and water was available at all times. All pigs were weighed at the beginning of each period to calculate feed allowance during the following period, and all pigs were weighed at the conclusion of the experiment.

Sample Collection

The initial 5 d of each period was considered the adaptation phase to the diets, and ileal digesta were collected for 9 h on d 6 and 7 following procedures explained by Stein et al. (1998). In brief, cannulas were opened and cleaned, a 232 mL capacity plastic bag was secured to the cannula barrel by a cable tie, and ileal digesta flowing into the bag were collected. Bags were removed when they were filled with ileal digesta, or at least every 30 min, and immediately stored at –20 °C to prevent bacterial degradation of AA in the ileal digesta.

Chemical Analysis

Each pork product was subsampled at the start of the experiment, and a sample of the N-free diet was collected at the time of mixing. At the conclusion of each experimental period, ileal digesta samples were thawed at room temperature and mixed within animal and diet and a subsample was collected. Prior to chemical analysis, pork products and ileal digesta samples were lyophilized and finely ground. Pork products, ileal digesta, and the N-free diet were analyzed in duplicate for DM (Method 930.15; AOAC Int., 2007) and AA [Method 982.30 E (a, b, c); AOAC Int., 2007]. The N-free diet and ileal digesta samples were analyzed in duplicate for titanium (Myers et al., 2004), and CP was analyzed in those samples using the combustion procedure (Method 990.03; AOAC Int., 2007) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI). All the pork products were analyzed in triplicate for CP using the Kjeldahl method (Method 984.13; AOAC Int., 2007) on a KjeltecTM 8400 (FOSS Inc., Eden Prairie, MN). Pork products and the N-free diet were analyzed in triplicate for ash at 600 ºC for 12 h (Method 942.05; AOAC Int., 2007), and for GE using an isoperibol bomb calorimeter (Model 6400; Parr Instruments, Moline, IL) with benzoic acid as the standard for calibration. Acid hydrolyzed ether extract (**AEE**) was analyzed in the N-free diet and the pork products using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY).

Calculations

Apparent ileal digestibility (**AID**) of CP and AA in diets was calculated using equation [1] (Stein et al., 2007).

$$\text{AID (\%)} = 1 - [(AA_d/AA_f) \times (Ti_f/Ti_d)] \times 100, \quad \textbf{[1]}$$

where AID is the apparent ileal digestibility of an AA (%), $\mathbf{AA_d}$ is the concentration of that AA in the ileal digesta DM, $\mathbf{AA_f}$ is the AA concentration of that AA in the diet DM, $\mathbf{Ti_f}$ is the titanium concentration in the diet DM, and $\mathbf{Ti_d}$ is the titanium concentration in the ileal digesta DM. The AID of 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 N-free diet using equation [2] (Stein et al., 2007).

$$IAA_{end} = [AA_d \times (Ti_f/Ti_d)], \quad \textbf{[2]}$$

where $\mathbf{IAA_{end}}$ is the basal endogenous loss of an AA (mg/kg DMI). The basal endogenous loss of CP was determined using the same equation.

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

$$SID = [(AID + IAA_{end})/AA_f], \qquad \textbf{[3]}$$

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

The concentration of SID AA (g/kg) in each ingredient was calculated by multiplying the SID value (%) for each AA by the concentration (g/kg) of that AA in the ingredient. This value was then divided by the concentration of CP in the ingredient to calculate digestible indispensable AA content (mg) in 1 g protein (Cervantes-Pahm et al., 2014). The digestible indispensable AA reference ratios were calculated for each ingredient using the following equation [4] (FAO, 2013):

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

Separate ratios were calculated using the reference protein for children from 6 mo to 3 yr, and children older than 3 yr, adolescents, and adults (FAO, 2013). The DIAAS values were calculated using the following equation [5] (FAO, 2013):

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

Statistical Analysis

Normality of the data was tested by generating studentized residuals from each analysis. Outliers were removed until the Shapiro-Wilk's test reached $P > 0.05$ and studentized residuals were within ± 3 standard deviations. Data were analyzed using PROC MIXED of SAS (SAS Institute Inc., Cary, NC) in a randomized complete block design with the pig as the experimental unit. Diet was the main effect and pig and period were random effects in the statistical model determining differences in SID of AA among ingredients. Treatment means were calculated using the LSMEANS statement, and if significant, means were separated using the PDIFF option of the MIXED procedure. Significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

All pigs remained healthy throughout the experiment and readily consumed their daily feed allowance.

Apparent Ileal Digestibility

The AID of most AA was not different between smoked and smoked-cooked bacon, with the exception that the AID of His and Trp was greater in smoked bacon than in smoked-cooked bacon (Table 3.5). The AID of Arg, Lys, Met, Phe, Ala, Gly, and Ser was greater ($P < 0.05$) in raw belly than in smoked bacon, whereas the AID of most AA did not differ between raw belly and smoked-cooked bacon. No differences in the AID of all AA was observed between conventionally cured and alternatively cured ham, but conventionally cured and alternatively cured ham had greater ($P < 0.05$) AID of all AA, except Tyr, than non-cured ham. The AID of all AA, except Gly, was not different among the 3 processing techniques for loin (i.e., medium, medium-well, and well-done), and the AID of most AA did not differ between the 2 bacon processing techniques and the 3 loin ingredients. Raw belly had greater ($P < 0.05$) AID of Arg, His, Ile, Phe, and Val than medium-well and well-done loins, whereas raw belly, smoked-cooked bacon, and medium loin had greater ($P < 0.05$) AID of all AA, except Met and Tyr, than non-cured ham.

Standardized Ileal Digestibility

The SID of His, Ile, Leu, Lys, Met, Phe, Trp, Asp, Gly, and Tyr was greater ($P < 0.05$) in raw belly than in smoked-cooked bacon (Table 3.6), but the SID of all AA, except His, was not different between smoked and smoked-cooked bacon, and the SID of all AA, except Arg, His, Ala, and Gly was not different between smoked bacon and raw belly. Conventionally cured and alternatively cured ham had greater ($P < 0.05$) SID of all AA, except Trp and Tyr, compared with non-cured. All other pork products had greater ($P < 0.05$) SID of all IAA, except Arg, compared with non-cured ham. No difference in the SID of AA was observed among the 3 processing techniques for loin. In addition, no differences were observed in the SID of all IAA, except His and Trp, among smoked bacon, smoked-cooked bacon, conventionally cured ham, alternatively cured ham, and the 3 loin ingredients.

DIAAS

For children from 6 mo to 3 yr (Table 3.7), loin heated to a medium degree of doneness had the greatest ($P < 0.05$) DIAAS followed by smoked-cooked bacon. Alternatively cured ham had a greater ($P < 0.05$) DIAAS value than the other 2 ham ingredients (conventionally cured and non-cured ham), but the conventionally cured and non-cured ham did not differ for DIAAS. Raw belly, smoked bacon, and medium-well and well-done loins were not different for DIAAS and had the least ($P < 0.05$) DIAAS values compared with the other pork products. The first limiting AA in all pork products was Val, with the exception that Trp was the first limiting AA for DIAAS in smoked-cooked bacon.

For children older than 3 yr, adolescents, and adults, smoked-cooked bacon had the greatest ($P < 0.05$) DIAAS value followed by pork loin cooked to a medium degree of doneness. The DIAAS for conventionally cured and non-cured ham did not differ, but alternatively cured ham had greater ($P < 0.05$) DIAAS than conventionally cured and non-cured ham. Raw belly, smoked bacon, and medium-well and well-done loins were not different for DIAAS and had the least ($P < 0.05$) DIAAS values compared with the other pork products. The first limiting AA for all pork products for DIAAS was Val regardless of the processing method.

Using DIAAS cut-off values, protein quality can be described as 'Excellent', if DIAAS is greater than 100% and 'Good', if DIAAS is between 75% and 99% (FAO, 2013). Based on these cut-off values, all pork products used in this experiment can be described as 'Excellent' quality proteins if consumed by children from 6 mo to 3 yr or by persons that are 3 yr or older.

DISCUSSION

The CP and AA composition in all pork products were generally within the range of published values (USDA, 2018). To our knowledge, the AA profile of smoked bacon and alternatively cured ham have not been reported, but when compared to published values for various cuts of uncooked bacon products and fully cooked ham traditionally cured with Prague powder, respectively, AA concentrations in the proteins used in this experiment were in agreement with published values.

Before consumption, pork products almost always undergo various forms of processing (Seman et al., 2018). Processing is primarily carried out to develop sensorial qualities and inhibit the activity of pathogenic microorganisms (Prestat et al., 2002; Gatellier et al., 2010; Van Hecke et al., 2014). The effect of cooking on the structure of meat proteins has been widely studied and the temperature and duration of cooking affect the extent of protein denaturation, oxidation, and aggregation (Tornberg, 2005; Bax et al., 2012; He et al., 2018). The cooking effect on protein and AA digestibility is not well known, but it has been reported that moderate protein denaturation occurs around 70 to 72 ºC exposing protein cleavage sites to proteolytic enzymes resulting in increased digestibility (Bax et al., 2012; Di Luccia et al., 2015; He et al., 2018). Hodgkinson et al. (2018) observed increased protein quality when beef steaks were boiled to an internal temperature of 71 ºC. However, protein modification at temperatures of 100 ºC or greater may result in protein oxidation and aggregation, which decrease digestibility (Santé-Lhoutellier et al., 2008; Bax et al., 2012; Li et al., 2017).

In contrast with published data, the digestibility of AA in the pork loins did not increase as the cooking temperature increased from 63 to 72 ºC, and the digestibility between the loin ingredients did not differ. However, the DIAAS decreased as temperature increased, which is likely the result of the lower concentration of digestible IAA in 1 g of protein for medium-well and well-done loin compared with medium loin. The DIAAS for medium loin was greater than for raw belly indicating that moderate heating may increase protein quality of meat.

Curing, another form of processing, is characterized by the addition of salt, sodium or potassium nitrate or nitrite, sugar, or seasonings and the resulting meat is a reddish-pink color (USDA-FSIS, 2016). Nitrate or nitrite in combination with sodium chloride is largely used as a curing agent to inhibit the growth of *Clostridium botulinum* due to its antioxidant properties (Van Hecke et al., 2014). In this study, the conventionally cured ham was cured with sodium nitrite and the alternatively cured ham was cured with celery salt, which is naturally high in sodium nitrate. The resulting IAA digestibility in both products was greater than in non-cured ham, which was cooked, indicating that the addition of nitrate or nitrite and sodium chloride may inhibit protein oxidation and thereby protect the physical and chemical properties of the protein. Alternatively cured ham had a greater concentration of digestible IAA per g of protein compared with conventionally cured and non-cured ham resulting in a greater DIAAS value.

Bacon, a cured and smoked pork product, had the greatest value for DIAAS when it underwent cooking. However, the smoked-cooked bacon had decreased SID of IAA compared with the raw belly, which may be a result of over-heating during cooking. It is acknowledged that the internal temperature of the bacon was not monitored during cooking, potentially resulting in overcooking. However, the U.S. Department of Agriculture (**USDA**) and FSIS recognize the difficulty in determining the internal temperature of bacon due to the thickness of the product and have suggested that if bacon is cooked crisp, a safe internal temperature has been reached (USDA-FSIS, 2013). The decreased SID of IAA in smoked-cooked bacon did not negatively affect the DIAAS because of the greater IAA content per g of protein.

Regardless of processing method, all pork products evaluated in this experiment had DIAAS values greater than 100% confirming the high quality of pork protein. Dairy proteins, fish proteins, and animal protein hydrolysates also have DIAAS values greater than 100%, indicating that animal proteins, in general, are high quality proteins (Shaheen et al., 2016; Mathai et al., 2017; Bindari et al., 2018). DIAAS values greater than 100% indicate that high quality protein has the potential to complement low quality proteins (FAO, 2013). Cereal grains contribute the majority of energy in human diets, however, the notably limiting levels of Lys in cereal grains make them low quality proteins (Cervantes-Pahm et al., 2014). Pork products, naturally high in Lys, have the potential to complement cereal grains and balance the AA profile of a mixed diet.

In conclusion, pork products are high quality proteins with DIAAS values greater than 100% indicating that these proteins may complement low quality proteins to produce a diet adequate in all IAA. Results also indicated that various forms of processing did not negatively affect DIAAS and that curing and moderate heating of pork products may increase DIAAS.

TABLES

Table 3.1. Cooking procedure of the 9 pork ingredients

Ingredient	Processing information
Raw belly[1]	Uncooked, unprocessed pork belly.
Smoked bacon	Cured with water, salt, sugar, sodium erythorbate, and sodium nitrite. Smoked in a commercial industrial smokehouse cycle and then cooled and sliced.
Smoked-cooked bacon	Cured with water, salt, sugar, sodium erythorbate, and sodium nitrite. Smoked in a commercial industrial smokehouse cycle and then cooled and sliced. Sliced bacon was then fully cooked with a commercial microwave continuous cooking system.
Non-cured ham[2]	Fresh pork leg that was not processed with a curing solution. Cooked in a commercial smokehouse with no smoke cycle at 176.6 ºC until the largest ham reached an internal temperature of 73 to 74 ºC.
Alternatively cured ham[2]	Cured with celery salt/no added nitrite. Hams were injected with brine on d 1 via stitch pumping and placed in a large tub for 24 h. On d 2, hams were cooked in a commercial smokehouse with no smoke cycle at 176.6 ºC until the largest ham reached an internal temperature of 73 to 74 ºC.

Table 3.1 (cont.)

Conventionally cured ham[2]	Cured with a traditional pink Prague powder curing recipe. Hams were injected with brine on d 1 via stitch pumping and placed in a large tub for 24 h. On d 2, hams were cooked in a commercial smokehouse with no smoke cycle at 176.6 ºC until the largest ham reached an internal temperature of 73 to 74 ºC.
Medium loin[3]	Cooked to 63 ºC (medium) in a convection oven (SL-series, Southbend, Co., Fuquay-Varina, NC) at 149 ºC. Roasts were removed from the cooking cycle 5 ºC prior to reaching desired temperature and allowed to rest prior to being chilled in the cooler at 4 ºC.
Medium-well loin[3]	Cooked to 68 ºC (medium-well) in a convection oven (SL-series, Southbend, Co., Fuquay-Varina, NC) at 149 ºC. Roasts were removed from the cooking cycle 5 ºC prior to reaching desired temperature and allowed to rest prior to being chilled in the cooler at 4 ºC.
Well-done loin[3]	Cooked to 72 ºC (well-done) in a convection oven (SL-series, Southbend, Co., Fuquay-Varina, NC) at 149 ºC. Roasts were removed from the cooking cycle 5 ºC prior to reaching desired temperature and allowed to rest prior to being chilled in the cooler at 4 ºC.

[1]Purchased in accordance to the Institutional Meat Purchasing Specifications (IMPS #413; North American Meat Processors [NAMP], 2007).

[2]Hams were classified as boneless fresh pork leg, inside (IMPS #402F; NAMP, 2007).

[3]Loins were classified as fresh, boneless pork loin roast (IMPS #413; NAMP, 2007).

Table 3.2. Analyzed nutrient composition of pork ingredients and the N-free diet (as-fed basis)

Item	Raw belly	Bacon		Ham			Loin			N-free
		Smoked	Smoked-cooked	Non-cured	Alt. cured[1]	Conv. cured[2]	Medium	Medium-well	Well-done	
GE[3], kcal/kg	3,588	2,835	4,921	2,143	1,647	1,543	1,974	1,949	2,081	3,699
Dry matter, %	45.44	44.00	76.56	38.77	31.33	30.26	33.85	35.58	36.52	93.97
Crude protein, %	16.46	19.15	34.13	34.52	24.17	23.79	25.58	30.86	33.30	0.40
Ash, %	0.49	2.93	5.53	1.56	2.38	3.16	1.26	1.29	1.47	4.79
AAE[4], %	31.77	19.34	27.58	3.42	2.67	2.27	5.34	5.44	4.67	1.38
Indispensable AA, %										
Arg	1.09	1.20	2.63	2.31	1.66	1.54	1.89	1.94	2.09	0.01
His	0.59	0.76	1.51	1.38	1.05	1.00	1.17	1.25	1.27	0.01
Ile	0.77	0.88	1.87	1.79	1.30	1.21	1.47	1.50	1.63	0.01
Leu	1.25	1.45	3.10	2.93	2.12	1.97	2.39	2.42	2.64	0.03
Lys	1.41	1.61	3.36	3.24	2.32	2.17	2.65	2.69	2.94	0.02

Table 3.2 (cont.)

Met	0.42	0.39	0.80	1.00	0.63	0.56	0.80	0.83	0.89	0.00
Phe	0.66	0.76	1.64	1.51	1.09	1.01	1.22	1.25	1.34	0.02
Thr	0.73	0.83	1.76	1.64	1.17	1.09	1.34	1.36	1.48	0.02
Trp	0.16	0.24	0.38	0.46	0.34	0.31	0.40	0.40	0.44	0.02
Val	0.80	0.94	2.03	1.84	1.34	1.24	1.50	1.53	1.65	0.01
Total	7.87	9.05	19.08	18.09	13.01	12.09	14.84	15.17	16.36	0.15
Dispensable AA, %										
Ala	0.95	1.08	2.50	2.02	1.47	1.36	1.65	1.71	1.84	0.02
Asp	1.42	1.67	3.61	3.34	2.41	2.23	2.74	2.79	3.03	0.02
Cys	0.19	0.21	0.45	0.39	0.29	0.28	0.33	0.33	0.35	0.01
Glu	2.05	2.47	5.55	5.20	3.74	3.50	4.22	4.28	4.91	0.05
Gly	1.05	1.09	2.74	1.51	1.13	1.04	1.25	1.38	1.39	0.01
Pro	0.78	0.87	1.87	1.23	0.92	0.84	1.01	1.07	1.20	0.03
Ser	0.59	0.68	1.48	1.26	0.90	0.83	1.03	1.05	1.25	0.01
Tyr	0.71	0.81	1.65	1.66	1.21	1.15	1.39	1.45	1.52	0.01

Table 3.2 (cont.)

Total	7.74	8.87	19.85	16.62	12.07	11.24	13.61	14.06	15.49	0.16
Total AA, %	15.61	17.92	38.93	34.71	25.09	23.33	28.45	29.23	31.85	0.31

[1]Alt. cured = alternatively cured ham.

[2]Conv. cured = conventionally cured ham.

[3]GE = gross energy.

[4]AEE = acid hydrolysis ether extract.

Table 3.3. Ingredient composition of experimental diets (as-fed basis)[1]

Ingredient, %	Raw belly	Bacon		Ham			Loin			N-free
		Smoked	Smoked-cooked	Non-cured	Alt. cured[2]	Conv. cured[3]	Medium	Medium-well	Well-done	
Pork product	62.80	55.93	41.25	34.95	44.18	44.45	42.93	37.56	35.53	-
Cornstarch	25.07	29.70	39.60	43.85	37.62	37.44	38.46	42.08	43.45	67.40
Solka floc	1.49	1.76	2.35	2.60	2.23	2.22	2.28	2.50	2.58	4.00
Soybean oil	1.49	1.76	2.35	2.60	2.23	2.22	2.28	2.50	2.58	4.00
Limestone	0.19	0.22	0.29	0.33	0.28	0.28	0.29	0.31	0.32	0.50
Dicalcium phosphate	0.89	1.06	1.41	1.56	1.34	1.33	1.37	1.50	1.55	2.40
Sodium chloride	0.15	0.18	0.24	0.26	0.22	0.22	0.23	0.25	0.26	0.40
Magnesium oxide	0.04	0.04	0.06	0.07	0.06	0.06	0.06	0.06	0.06	0.10
Potassium carbonate	0.15	0.18	0.24	0.26	0.22	0.22	0.23	0.25	0.26	0.40

Table 3.3 (cont.)

Sucrose	7.44	8.81	11.75	13.01	11.16	11.11	11.41	12.49	12.89	20.00
Titanium dioxide	0.19	0.22	0.29	0.33	0.28	0.28	0.29	0.31	0.32	0.50
Vitamin mineral premix[4]	0.11	0.13	0.18	0.20	0.17	0.17	0.17	0.19	0.19	0.30

[1] All diets were formulated to contain approximately 16% crude protein, as-fed basis.

[2]Alt. cured = alternatively cured ham.

[3]Conv. cured = conventionally cured ham.

[4] The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 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 B_{12}, 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 3.4. Calculated nutrient composition of experimental diets (dry matter basis)[1]

Item	Raw belly	Bacon		Ham			Loin			N-free[4]
		Smoked	Smoked-cooked	Non-cured	Alt. cured[2]	Conv. cured[3]	Medium	Medium-well	Well-done	
Dry matter, %	63.49	66.02	86.79	74.68	66.29	65.65	68.16	72.03	73.56	93.97
Crude protein, %	25.64	24.57	18.69	21.63	24.29	24.54	23.65	22.34	21.87	0.43
Indispensable AA, %										
Arg	1.08	1.02	1.25	1.08	1.11	1.04	1.19	1.01	1.01	0.01
His	0.58	0.64	0.72	0.65	0.70	0.68	0.74	0.65	0.61	0.01
Ile	0.76	0.75	0.89	0.84	0.87	0.82	0.93	0.78	0.79	0.01
Leu	1.24	1.23	1.47	1.37	1.41	1.33	1.51	1.26	1.28	0.03
Lys	1.39	1.36	1.60	1.52	1.54	1.47	1.67	1.40	1.42	0.02
Met	0.41	0.33	0.38	0.47	0.42	0.38	0.51	0.43	0.43	0.00
Phe	0.65	0.64	0.78	0.70	0.73	0.69	0.77	0.65	0.65	0.02
Thr	0.72	0.70	0.84	0.77	0.78	0.74	0.84	0.71	0.72	0.02
Trp	0.16	0.20	0.18	0.21	0.23	0.21	0.25	0.21	0.21	0.02

Table 3.4 (cont.)

Val	0.79	0.79	0.96	0.86	0.89	0.84	0.94	0.80	0.79	0.01
Total	7.78	7.67	9.07	8.47	8.67	8.19	9.35	7.91	7.90	0.15
Dispensable AA, %										
Ala	0.94	0.92	1.19	0.94	0.98	0.92	1.04	0.89	0.89	0.02
Asp	1.41	1.41	1.72	1.57	1.60	1.51	1.72	1.45	1.46	0.02
Cys	0.18	0.18	0.22	0.18	0.20	0.19	0.21	0.17	0.17	0.01
Glu	2.03	2.09	2.64	2.43	2.50	2.37	2.66	2.23	2.37	0.05
Gly	1.04	0.92	1.30	0.71	0.75	0.70	0.79	0.72	0.67	0.01
Pro	0.77	0.73	0.89	0.58	0.62	0.57	0.64	0.56	0.58	0.03
Ser	0.59	0.58	0.70	0.59	0.60	0.56	0.65	0.55	0.60	0.01
Tyr	0.70	0.68	0.78	0.78	0.81	0.78	0.88	0.75	0.73	0.01
Total	7.66	7.51	9.43	7.78	8.05	7.61	8.57	7.33	7.48	0.16
Total AA, %	15.44	15.18	18.50	16.25	16.72	15.80	17.92	15.24	15.38	0.31

[1]Diets were formulated to contain approximately 16% crude protein on an as-fed basis.

[2]Alt. cured = alternatively cured ham.

[3]Conv. cured = conventionally cured ham.

Table 3.4 (cont.)

[4]The nutrient composition of the N-free diet was analyzed.

Table 3.5. Apparent ileal digestibility (AID) of amino acid (AA) in ingredients[1]

Item	Raw	Bacon		Ham			Loin			Pooled	*P*-value
	belly	Smoked	Smoked-cooked	Non-cured	Alt. cured[2]	Conv. cured[3]	Medium	Medium-well	Well-done	SEM	
Indispensable AA, %											
Arg	95.0[a]	93.0[de]	94.8[ab]	92.7[e]	94.0[abcd]	94.1[abc]	94.2[abc]	93.8[bcd]	93.6[cde]	0.44	<0.001
His	94.6[a]	93.2[ab]	91.7[c]	89.9[d]	91.8[bc]	92.5[bc]	92.6[bc]	92.7[bc]	92.2[bc]	0.61	<0.001
Ile	92.7[a]	91.3[abc]	91.7[abc]	89.3[d]	91.9[abc]	92.3[ab]	91.7[abc]	90.7[cd]	91.0[bc]	0.63	0.003
Leu	92.7[a]	91.4[abc]	92.0[ab]	90.0[c]	92.2[ab]	92.5[ab]	92.1[ab]	91.1[bc]	91.3[abc]	0.61	0.016
Lys	94.3[a]	93.0[bc]	93.1[b]	91.8[c]	93.3[ab]	93.9[ab]	94.0[ab]	93.1[ab]	93.3[ab]	0.52	0.018
Met	95.9[a]	94.8[bc]	94.7[bc]	93.9[c]	95.2[ab]	95.3[ab]	95.5[ab]	95.1[ab]	95.1[ab]	0.39	0.017
Phe	91.2[a]	89.4[bc]	90.5[ab]	87.9[c]	90.4[ab]	90.7[ab]	90.3[ab]	89.4[bc]	89.2[bc]	0.71	0.012
Thr	87.8[a]	85.6[abc]	87.3[ab]	83.5[c]	87.1[ab]	87.0[ab]	87.0[ab]	85.5[bc]	85.5[abc]	0.91	0.007
Trp	88.9[ab]	89.4[a]	87.1[b]	84.9[c]	87.1[b]	87.5[b]	88.3[ab]	87.9[ab]	87.7[ab]	0.92	0.002
Val	91.0[a]	89.4[ab]	90.5[ab]	87.1[c]	90.1[ab]	90.5[ab]	89.8[ab]	88.8[bc]	88.9[bc]	0.79	0.005
Mean	92.8[a]	91.2[b]	91.8[ab]	89.6[c]	91.8[ab]	92.1[ab]	91.9[ab]	91.2[b]	92.1[b]	0.61	0.010

Table 3.5 (cont.)

Dispensable AA, %											
Ala	91.4^{ab}	89.3^{cd}	91.5^{a}	88.5^{d}	90.9^{abc}	91.4^{ab}	90.9^{abc}	89.8^{bcd}	90.1^{abcd}	0.70	0.005
Asp	90.7^{a}	89.0^{abc}	89.7^{ab}	81.2^{e}	88.7^{abcd}	89.1^{ab}	88.3^{bcd}	86.9^{cd}	86.6^{d}	0.91	<0.001
Cys	78.5^{a}	74.2^{abc}	76.4^{ab}	63.0^{d}	75.3^{ab}	75.5^{ab}	72.7^{bc}	69.7^{c}	70.7^{c}	2.05	<0.001
Glu	92.6^{a}	91.3^{a}	92.6^{a}	89.2^{b}	91.9^{a}	92.8^{a}	91.6^{a}	91.4^{a}	92.1^{a}	0.80	0.016
Gly	87.2^{a}	79.2^{bcd}	86.5^{a}	72.1^{e}	81.6^{b}	78.9^{bcd}	80.0^{bc}	77.2^{cd}	75.6^{de}	1.58	<0.001
Ser	88.3^{a}	86.1^{bc}	88.0^{ab}	82.6^{d}	86.2^{abc}	86.3^{abc}	86.4^{abc}	84.9^{c}	86.1^{abc}	0.91	<0.001
Tyr	93.2^{a}	91.9^{abc}	91.2^{bc}	90.5^{c}	91.8^{abc}	92.4^{ab}	92.3^{ab}	92.2^{ab}	91.9^{abc}	0.64	0.062
Mean	90.3^{a}	87.6^{bc}	89.5^{ab}	84.3^{d}	88.9^{abc}	89.1^{abc}	88.5^{abc}	87.4^{c}	87.7^{bc}	0.87	<0.001
Total AA, %	91.6^{a}	89.4^{b}	90.7^{ab}	87.1^{c}	90.4^{ab}	90.7^{ab}	90.3^{ab}	89.4^{b}	89.5^{b}	0.73	<0.001

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

[1]Data are least squares means of 10 observations per treatment except for smoked bacon, conventionally cured, alternatively cured, and non-cured ham, and medium loin that have 9 observations per treatment and for raw belly, medium-well, and well-done loin that have 8 observations per treatment.

[2]Alt. cured = alternatively cured ham.

Table 3.5 (cont.)

[3]Conv. cured = conventionally cured ham.

Table 3.6. Standardized ileal digestibility (SID) of amino acid (AA) in ingredients[1,2]

Item	Raw belly	Bacon		Ham			Loin			Pooled SEM	*P*-value
		Smoked	Smoked-cooked	Non-cured	Alt. cured[3]	Conv. cured[4]	Medium	Medium-well	Well-done		
Indispensable AA, %											
Arg	100.3[a]	98.6[bc]	99.4[ab]	98.0[c]	99.2[b]	99.6[ab]	99.0[bc]	99.5[ab]	99.3[ab]	0.44	0.008
His	98.5[a]	96.7[b]	94.8[c]	93.4[d]	95.0[c]	95.8[bc]	95.6[bc]	96.2[bc]	95.9[bc]	0.61	<0.001
Ile	97.8[a]	96.6[ab]	96.1[b]	93.9[c]	96.4[ab]	97.1[ab]	95.9[b]	95.7[b]	96.0[b]	0.63	0.001
Leu	98.0[a]	96.8[ab]	96.5[b]	94.8[c]	96.9[ab]	97.4[ab]	96.5[b]	96.3[b]	96.5[ab]	0.61	0.011
Lys	98.5[a]	97.2[ab]	96.7[bc]	95.7[c]	97.1[b]	97.8[ab]	97.4[ab]	97.3[ab]	97.4[ab]	0.52	0.009
Met	98.0[a]	97.4[ab]	97.0[b]	95.8[c]	97.3[ab]	97.7[ab]	97.3[ab]	97.1[ab]	97.1[ab]	0.39	0.007
Phe	97.6[a]	95.9[ab]	95.8[b]	93.9[c]	96.1[ab]	96.8[ab]	95.7[b]	95.8[b]	95.7[b]	0.71	0.013
Thr	97.8[a]	95.8[ab]	95.8[ab]	92.8[c]	96.3[ab]	96.7[ab]	95.5[b]	95.6[ab]	95.5[ab]	0.91	0.010
Trp	99.0[a]	97.2[ab]	95.7[bc]	92.2[d]	94.0[cd]	94.9[c]	94.5[c]	95.4[bc]	95.1[c]	0.92	<0.001
Val	97.0[a]	95.4[ab]	95.4[ab]	92.6[c]	95.4[ab]	96.1[ab]	94.8[b]	94.8[b]	94.9[b]	0.79	0.005
Mean	98.3[a]	96.8[b]	96.5[b]	94.7[c]	96.7[b]	97.3[ab]	96.5[b]	96.6[b]	96.6[b]	0.61	0.005

Table 3.6 (cont.)

Dispensable AA, %											
Ala	97.6^{a}	95.7^{bc}	96.4^{ab}	94.6^{c}	96.9^{ab}	97.7^{a}	96.5^{ab}	96.4^{abc}	96.6^{ab}	0.70	0.029
Asp	97.3^{a}	95.6^{ab}	95.1^{bc}	87.2^{e}	94.5^{bcd}	95.3^{abc}	93.7^{bcd}	93.3^{cd}	93.0^{d}	0.91	<0.001
Cys	92.6^{a}	88.7^{ab}	88.4^{ab}	77.1^{c}	88.5^{ab}	89.1^{ab}	85.3^{b}	84.8^{b}	85.9^{b}	2.05	<0.001
Glu	97.8^{a}	96.3^{ab}	96.5^{ab}	93.5^{c}	96.2^{ab}	97.3^{ab}	95.5^{b}	96.1^{ab}	96.6^{ab}	0.80	0.007
Gly	102.9^{a}	96.9^{cd}	99.0^{bc}	95.1^{d}	103.2^{a}	102.1^{ab}	100.8^{ab}	99.8^{abc}	99.8^{abc}	1.58	<0.001
Ser	99.1^{a}	96.9^{ab}	96.9^{ab}	93.2^{c}	96.7^{b}	97.5^{ab}	96.1^{b}	96.4^{b}	96.5^{b}	0.91	0.002
Tyr	98.0^{a}	96.8^{ab}	95.5^{bc}	94.8^{c}	95.9^{bc}	96.7^{ab}	96.2^{bc}	96.7^{ab}	96.5^{ab}	0.64	0.015
Mean	102.2^{a}	99.7^{b}	99.2^{b}	96.0^{c}	100.2^{ab}	101.1^{ab}	99.1^{b}	99.8^{b}	99.9^{b}	0.87	<0.001
Total AA, %	100.2^{a}	98.2^{b}	97.9^{b}	95.3^{c}	98.4^{b}	99.1^{ab}	97.8^{b}	98.1^{b}	98.2^{b}	0.73	<0.001

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

[1]Data are least squares means of 10 observations per treatment except for smoked bacon, conventionally cured, alternatively cured, and non-cured ham, and medium loin that have 9 observations per treatment and for raw belly, medium-well, and well-done loin that have 8 observations per treatment.

Table 3.6 (cont.)

[2]Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses (g/kg of dry matter intake) AA were as follows: crude protein, 14.99; Arg, 0.58; His, 0.22; Ile, 0.39; Leu, 0.66; Lys, 0.58; Met, 0.09; Phe, 0.42; Thr, 0.72; Trp, 0.16; Val, 0.48; Ala, 0.58; Asp, 0.94; Cys, 0.26; Glu, 1.05; Gly, 1.63; Pro, 3.67; Ser, 0.63; Tyr, 0.34.

[3]Alt. cured = alternatively cured ham.

[4]Conv. cured = conventionally cured ham.

Table 3.7. Digestible indispensable amino acid scores (DIAAS) for the 9 meat ingredients[1]

Item	Raw	Bacon		Ham			Loin			Pooled	P-value
	belly	Smoked	Smoked-cooked	Non-cured	Alt. cured[2]	Conv. cured[3]	Medium	Medium-well	Well-done	SEM	
DIAA reference ratio											
His	1.76	1.92	2.10	1.87	2.06	2.01	2.19	1.96	1.83		
Ile	1.43	1.39	1.64	1.52	1.62	1.54	1.73	1.45	1.47		
Leu	1.13	1.11	1.33	1.22	1.29	1.22	1.37	1.15	1.16		
Lys	1.48	1.44	1.67	1.57	1.63	1.56	1.77	1.49	1.51		
SAA[4]	1.31	1.11	1.27	1.35	1.34	1.24	1.53	1.31	1.30		
AAA[5]	1.57	1.52	1.77	1.66	1.76	1.69	1.89	1.62	1.59		
Thr	1.40	1.34	1.60	1.42	1.50	1.42	1.61	1.36	1.38		
Trp	1.12	1.43	1.26	1.44	1.56	1.45	1.73	1.46	1.49		
Val	1.11	1.09	1.32	1.15	1.23	1.17	1.29	1.09	1.09		
DIAAS, %											

Table 3.7 (cont.)

Child (6 mo to 3 yr)[6]	111[e] (Val)	109[e] (Val)	126[b] (Trp)	115[d] (Val)	123[c] (Val)	117[d] (Val)	129[a] (Val)	109[e] (Val)	109[e] (Val)	0.99	<0.001
DIAA reference ratio											
His	2.20	2.40	2.62	2.33	2.58	2.51	2.74	2.45	2.29		
Ile	1.53	1.48	1.75	1.62	1.73	1.64	1.84	1.55	1.57		
Leu	1.23	1.20	1.44	1.32	1.39	1.32	1.48	1.24	1.26		
Lys	1.76	1.71	1.98	1.87	1.94	1.85	2.11	1.77	1.79		
SAA[2]	1.54	1.30	1.50	1.58	1.57	1.46	1.80	1.54	1.53		
AAA[3]	1.99	1.92	2.25	2.11	2.23	2.14	2.39	2.06	2.02		
Thr	1.73	1.66	1.98	1.76	1.86	1.76	2.00	1.69	1.71		
Trp	1.44	1.84	1.62	1.85	2.00	1.87	2.23	1.88	1.92		
Val	1.19	1.17	1.42	1.24	1.33	1.26	1.39	1.18	1.17		
DIAAS, %											

Table 3.7 (cont.)

Older child, adolescent, adult[7]	119[e] (Val)	117[e] (Val)	142[a] (Val)	124[d] (Val)	133[c] (Val)	126[d] (Val)	139[b] (Val)	118[e] (Val)	117[e] (Val)	1.08	<0.001

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

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

[2]Alt. cured = alternatively cured ham.

[3]Conv. cured = conventionally cured ham.

[4]SAA = sulfur amino acid.

[5]AAA = aromatic amino acid.

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

[7]DIAAS were calculated using the recommended AA scoring pattern for 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; sulfur AA, 23; aromatic AA, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

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CHAPTER 4: DIGESTIBLE INDISPENSABLE AMINO ACID SCORES FOR RAW AND COOKED MEAT PRODUCTS AT VARIOUS STAGES OF PROCESSING

ABSTRACT

An experiment was conducted to test the hypothesis that meat products have digestible indispensable amino acid scores (**DIAAS**) that are greater than 100%, and that different food processing methods (i.e., roasting, curing, fermenting, or drying) will increase the standardized ileal digestibility (**SID**) of amino acid (**AA**) and the DIAAS of the protein. Nine ileal-cannulated gilts (initial BW: 35.50 ± 3.77 kg) were randomly allotted to a 9 × 8 Youden square design with 9 diets and eight 7-d periods during which ileal digesta samples were collected for 9 h on d 6 and 7 to determine AA digestibility. Eight diets contained a single meat product (i.e., salami, bologna, beef jerky, raw ground beef, cooked ground beef, medium-rare ribeye roast, medium ribeye roast, and well-done ribeye roast) as the sole source of AA. A N-free diet was used to determine basal endogenous losses of crude protein (**CP**) and AA, and to enable calculation of SID of AA. The DIAAS for each meat product was calculated using the SID of AA and the Food and Agriculture Organization of the United Nations (**FAO**) established reference protein patterns. The DIAAS value for all meat products, except cooked ground beef and well-done ribeye roast, calculated for children from 6 mo to 3 yr was greater than 100. For children older than 3 yr, adolescents, and adults, all meat products, except cooked ground beef, had a DIAAS value greater than 100%. Medium ribeye roast and bologna had the greatest ($P < 0.05$) values for DIAAS and cooked ground beef had the least ($P < 0.05$) DIAAS value for both reference protein patterns. Valine was the limiting AA in salami and the 3 types of ribeye roasts for older children,

adolescents, and adults, whereas sulfur containing AA was limiting in beef jerky and Leu was limiting in bologna, raw ground beef, and cooked ground beef. The SID for most AA was not different among salami, bologna, beef jerky, and cooked ground beef, but SID values in these proteins were less ($P < 0.05$) than the SID of most AA in raw ground beef. Medium-rare ribeye roast had SID values for AA that were comparable to values calculated for raw ground beef and well-done ribeye roast, but greater ($P < 0.05$) than the SID of most indispensable AA in medium ribeye roast. In conclusion, meat products generally provide high quality protein with DIAAS values that are greater than 100 regardless of the processing method. However, data indicate that overcooking of meat may reduce the SID of AA and DIAAS of the end product.

Key words: amino acids, digestible indispensable amino acid scores, protein quality, digestibility, beef, meat processing

INTRODUCTION

In the 2013 report by the Food and Agriculture Organization of the United Nations (**FAO**), it was recommended that the Digestible Indispensable Amino Acid Score (**DIAAS**) replaces the Protein Digestibility Corrected Amino Acid Score (**PDCAAS**) as the method for protein quality evaluation in foods for humans (FAO, 2013). The growing pig has been recognized as an appropriate model for determining DIAAS (FAO, 2013). To calculate DIAAS, the apparent ileal digestibility (**AID**) of amino acid (**AA**) is determined and then corrected for the basal endogenous loss of AA, and the resulting values are described as standardized ileal digestibility (**SID**) values. These values are equivalent to the true ileal digestibility values that are needed to calculate DIAAS for foods for humans (Stein et al., 2007; FAO, 2013).

Dairy proteins have DIAAS values that are greater than plant proteins (Rutherfurd et al., 2015; Mathai et al., 2017); other animal proteins, such as fish and animal protein hydrolysates, also have greater DIAAS than plant proteins (Shaheen et al., 2016; Bindari et al., 2018), and the DIAAS for beef was recently determined to be greater than in plant proteins (Hodgkinson et al., 2018). Meat is a concentrated source of protein providing indispensable AA (**IAA**) in diets for humans, and in most cases, meat is either minimally or further processed prior to consumption (Seman et al., 2018). Hodgkinson et al. (2018) observed only minor differences in AA digestibility in beef topside steak (semitendinosus muscle of the hind leg) cooked by various techniques and to a common degree of doneness (internal temperature of 71 ºC). Further processing techniques may increase the digestibility of AA because of changes in the 3-dimentional structures of proteins (Tornberg, 2005; Santé-Lhoutellier et al., 2008).

To our knowledge, no DIAAS values have been reported for meat heated to various degrees of doneness commonly consumed by humans. Furthermore, the influence of processing (e.g., drying, curing, fermenting) on SID of IAA and DIAAS has yet to be determined. Therefore, the objectives of this research were to test the hypotheses that meat products have DIAAS values that are greater than 100 and that cooking of ground or unground meat increases the SID of AA and the DIAAS of the protein.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment.

Preparation of Ingredients

Eight meat products were collected and prepared for feeding pigs at the Meat Science Laboratory at North Dakota State University, Fargo, ND (Tables 4.1 and 4.2). These products included salami, bologna, beef jerky, beef ribeye roast (cooked to 3 degrees of doneness), raw ground beef, and cooked ground beef. All products were obtained from commercial sources that are international suppliers of meat and meat products. Salami, bologna, and beef jerky were processed in accordance with Appendix A of the Food Safety and Inspection Services (**FSIS**) Compliance Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products (USDA-FSIS, 2017). The beef ribeye roasts (Institutional Meat Purchase Specifications #112A; NAMP, 2007) represented 3 treatments that were cooked at 56 (medium-rare), 64 (medium), or 72 ºC (well-done). The raw ground beef was coarse ground and one portion was uncooked and the remaining ground beef was fully cooked (> 72 ºC), drained of grease, and chilled prior to packaging and freezing at North Dakota State University. At the University of Illinois, the beef jerky was chopped in a 4-quart commercial food processor (Waring, Stamford, CT), and the salami, bologna, and ribeye roasts were chopped in an 8-quart bowl chopper (Professional Processor Food Equipment, New Ulm, TX). All meat products were vacuum packaged before being shipped to the University of Illinois, where they were stored at -20 ºC until use.

Diets, Animals, Housing, and Feeding

Nine diets were formulated (Tables 4.3 and 4.4); 8 diets contained a single meat product as the only crude protein (**CP**) and AA containing ingredient. A N-free diet was also formulated and used to determine basal endogenous losses of CP and AA. Titanium dioxide was included in the N-free diet at 0.5% as an indigestible marker. Vitamins and minerals were also included in the N-free diet to meet or exceed current nutrient requirements for growing pigs (NRC, 2012). The meat products were combined daily with sufficient amounts of the N-free diet to provide approximately 16% CP on an as-fed basis.

Nine growing female pigs (Line 2, Pig Improvement Company, Hendersonville, TN) with an initial body weight of 35.50 ± 3.77 kg were surgically fitted with a T-cannula in the distal ileum using procedures adapted from Stein et al. (1998). Following surgery, pigs were allowed a 7-d recovery period and then allotted to a 9 × 8 Youden square design with 9 diets and eight 7-d periods. All pigs received each diet only once during the experiment, therefore, there were 8 replicate pigs per treatment. Pigs were housed in individual pens (2 × 3 m) that had partially slatted concrete flooring in an environmentally controlled room. Each pen was equipped with smooth plastic siding, a feeder, and a nipple drinker. At the start of the first period pigs weighed approximately 44.40 ± 2.06 kg.

Pigs were fed a daily amount of feed equivalent to 4% of BW in 2 equal meals that were provided at 0800 and 1700 h. All pigs were weighed at the beginning of each period to calculate feed allowance during the following period, and all pigs were weighed at the conclusion of the experiment. Water was available at all times throughout the experiment.

Sample Collection

The initial 5 d of each period was considered the adaptation phase to the diets with the following 2 d being used for ileal digesta collection. Ileal digesta collection began at 0800 and ceased at 1700 h each day following procedures explained by Stein et al. (1998). In brief, cannulas were opened and cleaned, a 232 mL capacity plastic bag was attached to the cannula barrel and secured by a cable tie, and ileal digesta flowing into the bag were collected. Bags were removed when they were filled with ileal digesta, or at least once every 30 min, and immediately stored at –20 °C to prevent bacterial degradation of AA in the ileal digesta.

Chemical Analysis

At the conclusion of each experimental period, ileal digesta samples were thawed at room temperature and mixed within animal and diet, and a subsample was collected. A subsample of each source of protein was collected at the start of the experiment, and a sample of the N-free diet was collected at the time of mixing. Ileal digesta and ingredient samples were lyophilized and finely ground prior to chemical analysis. Ingredients were analyzed in duplicate for dry matter (Method 930.15; AOAC Int., 2007), ash at 600 ºC for 12 h (Method 942.05; AOAC Int., 2007), and AA [Method 982.30 E (a, b, c); AOAC Int., 2007]. Ingredients were also analyzed in triplicate for CP using the Kjeldahl method by quantifying N and using a conversion factor of 6.25 to calculate CP (Method 984.13; AOAC Int., 2007) on a KjeltecTM 8400 (FOSS Inc., Eden Prairie, MN), for GE using an isoperibol bomb calorimeter (Model 6400; Parr Instruments, Moline, IL) with benzoic acid as the standard for calibration, and for acid hydrolyzed ether extract (**AEE**) using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System; Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor; Ankom Technology, Macedon, NY). The N-free diet and ileal digesta samples were analyzed in duplicate for DM and AA as explained for the ingredients. Crude protein was determined in the N-free diet and in ileal digesta samples by the combustion procedure (Method 990.03; AOAC Int., 2007) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI). The N-free diet and ileal digesta samples were also analyzed in duplicate for titanium (Myers et al., 2004).

Calculations

The AID of CP and AA was calculated for all diets as previously explained by Stein et al. (2007). Values for AID of CP and AA were corrected for the basal endogenous loss of CP and each AA to calculate the SID of CP and AA (Stein et al., 2007). The SID of CP and AA calculated in each diet also represented the SID of the ingredient.

The concentration of SID AA (g/kg) in each ingredient was calculated by multiplying the SID value (%) for each AA by the concentration (g/kg) of that AA in the ingredient, and this value was divided by the concentration of CP in the ingredient to calculate digestible indispensable AA content (mg) in 1 g protein (Cervantes-Pahm et al., 2014; Mathai et al., 2017). The digestible indispensable AA reference ratios were calculated for each ingredient using the following equation [4] (FAO, 2013):

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

[4]

Separate ratios were calculated using the reference protein for children from 6 mo to 3 yr, and children older than 3 yr, adolescents, and adults (FAO, 2013). The DIAAS values were calculated using the following equation [5] (FAO, 2013):

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

Statistical Analysis

Studentized residuals from each analysis were generated and used to test normality of data. Outliers were removed until the Shapiro-Wilk's test reached $P > 0.05$ and studentized residuals were within ± 3 standard deviations. Data were analyzed using PROC MIXED of SAS (SAS Institute Inc., Cary, NC) in a randomized complete block design with the pig as the experimental unit. The statistical model to determine differences in SID of AA among ingredients included diet as the main effect and pig and period as random effects. Treatment means were calculated using the LSMEANS statement, and if significant, means were separated using the PDIFF option of the MIXED procedure. Significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

All pigs remained healthy throughout the experiment and readily consumed their daily feed allowance.

Apparent Ileal Digestibility

Among salami, bologna, and beef jerky, the AID of His, Trp, and Gly was greater ($P < 0.05$) in bologna than in salami, whereas the AID of most other AA did not differ (Table 4.5). Similarly, the AID of most AA did not differ among the 3 ribeye roast ingredients (i.e., medium-rare, medium, and well-done), with the exception that the AID of His was greater ($P < 0.05$) in medium-rare ribeye roast than in well-done ribeye roast. The AID of all AA was greater ($P < 0.05$) in raw ground beef than in cooked ground beef, except that there was no difference in the AID of Arg and Trp.

Bologna and medium-rare ribeye roast had greater ($P < 0.05$) AID of total IAA than cooked ground beef, and AID of total dispensable AA was greater ($P < 0.05$) in bologna and beef jerky than in cooked ground beef. The AID of Asp, Cys, and Gly was greater ($P < 0.05$) in bologna and raw ground beef than in the 3 ribeye roast ingredients; raw ground beef had greater ($P < 0.05$) AID of Leu, Met, Phe, and Thr than bologna, and greater ($P < 0.05$) AID of His and Thr than medium-rare ribeye roast. The AID of Lys was greater ($P < 0.05$) in raw ground beef than in cooked ground beef and beef jerky, but no differences were observed between beef jerky and the other ingredients. The AID of total AA was greater ($P < 0.05$) in bologna, beef jerky, and raw ground beef than in cooked ground beef and well-done ribeye roast.

Standardized Ileal Digestibility

Among salami, bologna, and beef jerky, the SID of all AA did not differ (Table 4.6). Medium-rare ribeye roast had greater ($P < 0.05$) SID of most AA compared with medium ribeye roast with the exception that no difference was observed in the SID of Ile, Met, Trp, Asp, Cys, and Glu. Well-done ribeye roast had greater ($P < 0.05$) SID of Arg, Thr, Trp, Ala, Gly, and Ser than medium ribeye roast, but the SID of all AA, except Asp, did not differ between well-done and medium-rare ribeye roasts. Raw ground beef had greater ($P < 0.05$) SID of His, Leu, Lys, Met, Phe, Asp, Cys, and Tyr than cooked ground beef, but cooked ground beef had greater ($P < 0.05$) SID of Arg than raw ground beef.

Raw ground beef had the greatest ($P < 0.05$) SID of Cys among all ingredients. The SID of Thr and Trp was greater ($P < 0.05$) in raw and cooked ground beef than in medium ribeye roast, raw ground beef and medium-rare ribeye roast had greater ($P < 0.05$) SID of His, Leu, Met, and Phe than salami, and raw ground beef had greater ($P < 0.05$) SID of His, Leu, and Lys than all other ingredients except medium-rare ribeye roast. The SID of Arg and His was greater ($P < 0.05$) in medium-rare ribeye roast than in beef jerky, and medium-rare ribeye roast had greater ($P < 0.05$) SID of His, Leu, Met, Phe, and Tyr than salami, whereas the only difference between medium-rare ribeye roast and bologna was the SID of Tyr. The SID of all AA, except Trp, did not differ among salami, bologna, beef jerky, and medium ribeye roast. Raw ground beef had greater ($P < 0.05$) SID of total IAA than all other ingredients except medium-rare and well-done ribeye roasts. The SID of total dispensable AA was greater ($P < 0.05$) in medium-rare and well-done ribeye roasts than in raw ground beef and greater ($P < 0.05$) in cooked ground beef than in medium ribeye roast. The SID of total AA was greater ($P < 0.05$) in medium-rare ribeye roast than in medium ribeye roast.

DIAAS

For children from 6 mo to 3 yr (Table 4.7), medium ribeye roast and bologna had the greatest ($P < 0.05$) DIAAS value and cooked ground beef had the least ($P < 0.05$) DIAAS value. The DIAAS for raw ground beef was less ($P < 0.05$) than for medium ribeye roast and bologna, but greater ($P < 0.05$) than for all other ingredients, whereas the DIAAS for salami was less ($P < 0.05$) than for medium ribeye roast, bologna, and raw ground beef. Beef jerky and medium-rare ribeye roast were not different, but both had greater ($P < 0.05$) DIAAS than well-done ribeye roast and cooked ground beef, and the DIAAS for well-done ribeye roast was greater ($P < 0.05$) than for cooked ground beef.

For children older than 3 yr, adolescents, and adults, medium ribeye roast and bologna had the greatest ($P < 0.05$) value for DIAAS and cooked ground beef had the least ($P < 0.05$) DIAAS value. Raw ground beef, salami, and beef jerky were not different, but these proteins had DIAAS values that were greater ($P < 0.05$) than medium-rare ribeye roast, which had a greater ($P < 0.05$) DIAAS than well-done ribeye roast.

For DIAAS calculated for both children from 6 mo to 3 yr and children older than 3 yr, adolescents, and adults, the first limiting AA in the 3 types of ribeye roast ingredients was Val, with the exception that Leu was the first limiting AA for DIAAS in well-done ribeye roast. The first limiting AA in raw and cooked ground beef and in bologna was Leu for both children from 6 mo to 3 yr and children older than 3 yr, adolescents, and adults, with the exception that Trp was the first limiting AA in raw ground beef for children from 6 mo to 3 yr. The first limiting

AA for children from 6 mo to 3 yr in beef jerky and salami was the sulfur containing AA. The sulfur containing AA was also the first limiting AA in beef jerky for children older than 3 yr, adolescents, and adults, whereas Val was the first limiting AA in salami.

Using DIAAS cut-off values, protein quality can be described as 'Excellent', if DIAAS is greater than 100% and 'Good', if DIAAS is between 75% and 99% (FAO, 2013). Based on these cut-off values, all meat products used in this experiment, except cooked ground beef, are described as 'Excellent' quality proteins if consumed by children older than 3 yr, adolescents, and adults. The 'Good' quality of cooked ground beef is attributed to the inadequate amount of Leu to meet the requirement of children older than 3 yr, adolescents, and adults. For children 6 mo to 3 yr, well-done ribeye roast and cooked ground beef are 'Good' quality proteins due to the limiting amount of Leu, but all other proteins are 'Excellent' quality.

DISCUSSION

Global undernutrition is estimated to affect 815 million people, or 11% of the global population (FAO et al., 2017). For children, stunting is considered the best available measurement of chronic malnutrition, and globally about 1 in 4 children under the age of 5 are affected (Semba et al., 2016; FAO et al., 2017). Stunting before the age of 2 is associated with increased morbidity and mortality and impaired cognitive and motor development, with 14.7% of child deaths attributed to stunting (Black et al., 2013). In addition, stunting has the potential to decrease economic development of entire nations, as indicated by the high prevalence of stunting in developing countries (FAO et al., 2017).

Sub-Saharan Africa and Southern Asia have rates of stunting that are among the highest in the world (FAO et al., 2017). Diets in these regions are largely composed of cereal grains, such as sorghum, wheat, rice, or maize, that are poor sources of IAA, especially limiting in Lys (Cervantes-Pahm et al., 2014; Shaheen et al., 2016; FAO et al., 2017; Abelilla et al., 2018). There is a strong association between stunting and low dietary intakes of quality proteins (Ghosh et al., 2012; Semba et al., 2016). Consequently, complementing cereal based diets with higher quality proteins may overcome IAA deficiencies. An excellent quality of dairy proteins was reported by Rutherfurd et al. (2015) and Mathai et al. (2017), and high DIAAS values for beef cooked to an internal temperature of 71 ºC were reported by Hodgkinson et al. (2018). Meat can be prepared by various techniques and to different degrees of doneness, but to our knowledge, there have been no studies conducted to determine DIAAS values for meat that have undergone extensive processing (e.g., drying, curing, fermenting) or the effects of varying degrees of doneness on DIAAS.

In the current experiment, concentrations of CP and AA in all meat products were generally within the range of published values (Bodwell and Anderson, 1986; USDA, 2018a), with the exception that, to our knowledge, there are no published values for concentrations of AA in beef jerky. The physicochemical characterization of the beef jerky used in this experiment was in accordance with the U.S. Department of Agriculture (**USDA**) and FSIS compliance guidelines for meat and poultry jerky (USDA-FSIS, 2014). When compared with similar dried beef products, the concentration of AA was slightly greater in beef jerky, which is likely due to variations in formulation (USDA, 2018a). For all meat products used in this experiment, the AA with the lowest concentration were Trp, Met, and His, in that order, regardless of the cooking method, which is in agreement with the literature (USDA, 2018a).

The protein quality of raw ground beef clearly decreased after cooking. The meat was fully browned using a Groen model IT-40 steam jacketed rendering kettle with bottom draw-off

(Groen Division/Dover Corporation, Elk Grove Village, IL). Steam circulated between the kettle and the outer wall, or jacket, and did not come into contact with the meat. The cooking of the ground beef was constantly monitored by a technician, and the ground beef was continuously turned with a spatula to prevent burning or over-browning. The USDA has set the safe minimum internal temperature for ground meats at 71.1 ºC (USDA, 2018b). In this experiment, the ground beef was considered fully cooked when the temperature was recorded above 72 ºC in 3 locations and when the batch was fully browned with no remaining pink/red meat, therefore, potentially leading to overcooking. Cooking meat to 70 ºC may enhance protein degradation by proteolytic enzymes due to the progressive effect heat has on protein denaturation (Gatellier et al., 2010; Bax et al., 2012; Bax et al., 2013). However, cooking at 100 ºC may lead to protein oxidation and the formation of carbonyl groups that may interact with free amino groups of non-oxidized AA leading to modification of AA, especially Lys, His, and Arg (Santé-Lhoutellier et al., 2008; Gatellier et al., 2010). In the present experiment, analyzed concentrations of Lys and His decreased after cooking, which indicates that the ground beef may have been overcooked. Cooking is important to inactivate pathogenic microorganism and is used to enhance the flavor of the meat (Santé-Lhoutellier et al., 2008). However, the decrease in digestibility and protein quality of cooked ground beef compared with raw ground beef indicates that heat damage may occur if the meat product is overcooked.

The observation that there was no difference in the SID of IAA between medium-rare and well-done ribeye roasts cooked to internal temperatures of 56 and 72 ºC, respectively, may be attributed to the use of finite temperature targets in combination with thermometers to obtain the desired degree of doneness, which reduced the risk of overcooking. In addition, ribeye roasts were cooked as whole-muscle, in-tact roasts, which reduced the protein exposure to oxygen and light. The lower SID of most IAA in medium ribeye roast compared with the medium-rare ribeye roast may be a result of the reduced amount of fat in the ingredient because increased fat increases SID of AA due to reduced rate of passage (Li and Sauer, 1994; Cervantes-Pahm and Stein, 2008). The reason the medium ribeye roast had the greatest DIAAS compared with all other meat products, despite lower SID values, is that this ingredient has a higher concentration of IAA compared with other ingredients included in the present experiment. Overall, cooking the ribeye roasts to different degrees of doneness had little impact on AA digestibility or protein quality.

Salami and bologna are cured, smoked sausages and in this experiment, both were fully cooked to the USDA recommended safe internal temperature of 71.1 ºC (USDA-FSIS, 2017; USDA, 2018b). Salami and bologna differ in that salami was subjected to a fermentation process to lower the pH and to improve shelf-life. Curing with sodium chloride and sodium nitrite is another method of meat preservation; however, with the advancement of modern preservation methods in developed countries, curing is more commonly used to obtain a reddish-pink color and induce a particular flavor in the end product (Heinz and Hautzinger, 2007). In developing countries, curing as a means of preserving meat is important in ensuring microbial stability and is essential in improving food distribution to malnourished populations (Weaver et al., 2014). Addition of ingredients that prevent oxidation during processing (i.e., nitrite and sodium chloride) may also aid in maintaining the structure of protein prior to digestion (Van Hecke et al., 2014). Beef jerky, another highly processed, cured meat product, is nutrient dense and shelf-stable due to removal of the majority of the moisture by drying (USDA-FSIS, 2014). For a product to be labeled jerky it must be dried to a moisture-protein ratio of less than 0.75:1 and have a water activity of less than 0.85, and prior to dehydration a temperature of 71.1 ºC must be reached, which enables the jerky to be kept at room temperature for up to 12 mo (USDA-FSIS, 2014). Salami, bologna, and beef jerky were all classified as excellent quality proteins with similar DIAAS values as raw ground beef further confirming that the processing techniques used to produce salami, bologna, and jerky do not negatively affect protein quality.

The high digestibility of the meat proteins in this experiment may be attributed to grinding. Grinding of whole-muscle proteins (i.e., in-tact ribeye roast) prior to consumption may have an impact similar to chewing, which increases in the surface area of the protein during digestion leading to more rapid digestion and absorption of AA (Rémond et al., 2007; Pennings et al., 2013). In contrast, grinding meat prior to cooking increases the surface area exposed to oxygen and light, and in combination with higher cooking temperatures there was a more negative impact on the DIAAS for cooked ground beef than for the ribeye roasts. For salami and bologna, the meat was ground prior to cooking, but these ingredients were also encased and heated from the outside, which resulted in more uniform cooking.

Hodgkinson et al. (2018) reported DIAAS values for beef from 80 to 99% as evaluated in the growing pig. Raw beef and roasted beef were reported with a DIAAS of 97 and 91%, respectively (Hodgkinson et al., 2018). Thus, the DIAAS values for raw ground beef and the 3 ribeye roast ingredients calculated in the present experiment are greater than the values by Hodgkinson et al. (2018). The beef prepared by Hodgkinson et al. (2018) was trimmed of all adipose and connective tissue, whereas the meat in the present experiment had 12 to 24% analyzed fat, except the cooked ground beef and beef jerky. In addition, in the experiment by Hodgkinson et al. (2018), ileal digesta were collected by anesthetizing and then euthanizing each pig and dissecting the terminal ileal from the body. Whereas, in the current experiment, ileal digesta were collected for 9 h on 2 consecutive days, which may have resulted in a more representative sampling of digesta. Lastly, the DIAAS reference pattern was not stated by Hodgkinson et al. (2018), which may explain DIAAS values being less than 100% because younger age groups have higher requirements for AA.

Rutherfurd et al. (2015) reported DIAAS values for whey and milk proteins determined in the growing rat ranging from 97 to 118%, and Mathai et al. (2017) reported DIAAS values for whey and milk proteins determined in the growing pig ranging from 123 to 141%. The DIAAS values for the meat proteins evaluated in the current experiment ranged from 99 to 130%, and according to FAO (2013), DIAAS greater than 100% indicate the potential of that protein to complement lower quality proteins. Specifically, meat proteins may complement cereal grains because of the high concentration of Lys in meat, whereas cereal grains are limiting in Lys (Cervantes-Pahm et al., 2014; Rutherfurd et al., 2015; Abelilla et al., 2018). In addition, most of the meat products included in this experiment had low concentration of Leu, but Leu is available in high concentration in corn and sorghum (Cervantes-Pahm et al., 2014), which indicates that a combination of cereal grains and meat will result in a high quality meal.

In conclusion, data from this experiment indicate that meat products generally provide high quality protein comparable to what has been reported for dairy proteins. Fermenting, curing, drying, or cooking meat to minimally safe internal temperatures does not negatively affect SID of AA or DIAAS. However, cooking meat that is ground or cooking meat with direct heat, for example on the stove top, may reduce protein quality, especially if overcooking occurs. The use of thermometers to monitor the internal temperature may aid in reducing the risk of overcooking, and therefore, contribute to maintaining the high quality of the meat protein.

TABLES

Table 4.1. Cooking procedure of the 8 meat ingredients

Ingredient	Processing information
Salami	Commercial grade, fully cooked, cured, and fermented. Ingredients included pork, pork hearts, water, salt, corn syrup, spices, dextrose, flavoring, sodium erythorbate, and sodium nitrite.
Bologna	Commercial grade, fully cooked, and cured. Ingredients included pork, water, beef, spices, salt, corn syrup, dextrose, sodium erythorbate, sodium nitrite, flavoring, and oleoresin of paprika.
Beef jerky	Cured with water, salt, brown sugar, sugar, monosodium glutamate, maple sugar, flavorings, and sodium nitrite.
Raw ground beef[1]	Fresh, unprocessed.
Cooked ground beef[1]	Fully browned in a Groen model IT-40 steam jacketed rendering kettle with bottom draw-off (Groen Division/Dover Corporation, Elk Grove Village, IL). The drippings were drained once the beef was fully browned.
Medium-rare ribeye roast[2]	Cooked to 56 °C (medium-rare) in a commercial smoke house at 121 °C. Ribeye roasts were removed from the cooking cycle 5 °C prior to reaching desired temperature and allowed to rest at room temperature.

Table 4.1 (cont.)

Medium ribeye roast[2]	Cooked to 64 ºC (medium) in a commercial smoke house at 121 ºC. Ribeye roasts were removed from the cooking cycle 5 ºC prior to reaching desired temperature and allowed to rest at room temperature.
Well-done ribeye roast[2]	Cooked to 72 ºC (well-done) in a commercial smoke house at 121 ºC. Ribeye roasts were removed from the cooking cycle 5 ºC prior to reaching desired temperature and allowed to rest at room temperature.

[1]All ground beef was 80% lean: 20% fat.

[2]All ribeye roasts were U.S. Department of Agriculture Select grade beef ribeye roast roll roast (Institutional Meat Purchase Specifications #112A; NAMP, 2007).

Table 4.2. Analyzed nutrient composition of meat ingredients and the N-free diet (as-fed basis)

Item	Salami	Bologna	Beef jerky	Ground beef		Ribeye roast			N-free
				Raw	Cooked	Medium-rare	Medium	Well-done	
GE[1], kcal/kg	2,153	2,751	3,180	3,024	2,291	2,507	2,863	3,538	3,688
Dry matter, %	34.83	40.25	66.11	40.72	35.69	37.52	42.75	48.25	94.04
Crude protein, %	17.08	11.93	48.86	18.11	30.56	24.40	26.42	26.27	0.45
Ash, %	3.42	3.21	9.12	0.80	0.92	0.89	1.44	0.76	2.70
AEE[2], %	12.15	20.26	1.94	18.57	9.36	14.41	13.99	23.47	3.44
Indispensable AA, %									
Arg	1.04	0.79	3.04	1.26	1.68	1.41	1.76	1.42	0.01
His	0.56	0.46	1.95	0.57	0.77	0.75	0.93	0.74	0.00
Ile	0.79	0.60	2.54	0.84	1.16	1.08	1.41	1.11	0.01
Leu	1.30	0.97	4.03	1.36	1.91	1.74	2.24	1.76	0.03
Lys	1.34	1.03	4.35	1.50	2.10	1.94	2.47	1.95	0.02
Met	0.33	0.26	0.90	0.45	0.60	0.55	0.74	0.56	0.01

Table 4.2 (cont.)

Phe	0.67	0.51	2.04	0.73	1.01	0.90	1.15	0.92	0.01
Thr	0.69	0.54	2.15	0.75	1.03	0.95	1.22	0.96	0.01
Trp	0.20	0.18	0.67	0.17	0.31	0.28	0.33	0.26	0.02
Val	0.86	0.65	2.56	0.90	1.27	1.12	1.45	1.16	0.01
Total	7.78	5.99	24.22	8.54	11.83	10.72	13.71	10.83	0.13
Dispensable AA, %									
Ala	0.94	0.71	2.86	1.19	1.57	1.23	1.58	1.26	0.01
Asp	1.45	1.12	4.48	1.60	2.19	1.95	2.50	1.95	0.02
Cys	0.20	0.15	0.53	0.20	0.25	0.24	0.31	0.25	0.00
Glu	2.23	1.73	7.82	2.44	3.44	3.02	3.90	2.93	0.03
Gly	0.93	0.75	2.17	1.49	1.76	1.06	1.27	1.09	0.01
Pro	0.72	0.61	1.86	1.00	1.25	0.90	1.08	0.92	0.02
Ser	0.55	0.44	1.54	0.63	0.80	0.71	0.91	0.72	0.01
Tyr	0.62	0.49	2.06	0.66	0.97	0.93	1.14	0.93	0.02
Total	7.64	6.00	23.30	9.21	12.23	10.03	12.70	10.04	0.12

Table 4.2 (cont.)

Total AA, %	15.42	12.00	47.52	17.75	24.06	20.75	26.41	20.88	0.25

[1]GE = gross energy.

[2]AEE = acid hydrolysis ether extract.

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

Ingredient, %	Salami	Bologna	Beef jerky	Ground beef		Ribeye roast			N-free
				Raw	Cooked	Medium-rare	Medium	Well-done	
Meat product	57.09	74.16	28.40	57.00	37.87	45.25	43.75	45.13	-
Cornstarch	28.93	17.42	48.26	28.98	41.86	36.92	37.90	37.01	67.40
Solka floc	1.72	1.03	2.86	1.72	2.49	2.19	2.25	2.19	4.00
Soybean oil	1.72	1.03	2.86	1.72	2.49	2.19	2.25	2.19	4.00
Limestone	0.21	0.13	0.36	0.22	0.31	0.27	0.28	0.27	0.50
Dicalcium phosphate	1.03	0.62	1.72	1.03	1.49	1.31	1.35	1.32	2.40
Sodium chloride	0.17	0.10	0.29	0.17	0.25	0.22	0.23	0.22	0.40
Magnesium oxide	0.04	0.03	0.07	0.04	0.06	0.05	0.06	0.05	0.10
Potassium carbonate	0.17	0.10	0.29	0.17	0.25	0.22	0.23	0.22	0.40

Table 4.3 (cont.)

Sucrose	8.58	5.17	14.32	8.60	12.43	10.95	11.25	10.97	20.00
Titanium dioxide	0.21	0.13	0.36	0.22	0.31	0.27	0.28	0.27	0.50
Vitamin mineral premix[2]	0.13	0.08	0.21	0.13	0.19	0.16	0.17	0.16	0.30

[1] All diets were formulated to contain approximately 16% crude protein on an as-fed basis.

[2] The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 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 B_{12}, 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. Calculated nutrient composition of experimental diets (dry matter basis)

Item	Salami	Bologna	Beef jerky	Ground beef		Ribeye roast			N-free[1]
				Raw	Cooked	Medium-rare	Medium	Well-done	
Dry matter, %	60.24	54.15	86.11	63.65	71.95	68.46	71.60	73.38	94.04
Crude protein[2], %	26.87	30.18	18.71	25.48	22.36	23.55	22.54	22.02	0.48
Indispensable AA, %									
Arg	0.98	1.09	1.00	1.13	0.88	0.93	1.08	0.87	0.01
His	0.53	0.63	0.64	0.51	0.41	0.50	0.57	0.45	0.00
Ile	0.75	0.83	0.84	0.75	0.61	0.71	0.86	0.68	0.01
Leu	1.23	1.32	1.33	1.21	1.00	1.15	1.37	1.08	0.03
Lys	1.27	1.42	1.43	1.34	1.10	1.28	1.51	1.20	0.02
Met	0.31	0.35	0.30	0.40	0.32	0.36	0.45	0.35	0.01
Phe	0.64	0.69	0.67	0.65	0.53	0.60	0.70	0.56	0.01
Thr	0.66	0.74	0.71	0.68	0.54	0.63	0.75	0.59	0.01
Trp	0.19	0.25	0.22	0.16	0.16	0.18	0.20	0.16	0.02

Table 4.4 (cont.)

Val	0.81	0.89	0.85	0.81	0.67	0.74	0.89	0.71	0.01
Total	7.37	8.21	7.99	7.65	6.23	7.08	8.37	6.66	0.13
Dispensable AA, %									
Ala	0.90	0.97	0.94	1.07	0.83	0.82	0.97	0.77	0.01
Asp	1.37	1.54	1.48	1.43	1.15	1.29	1.53	1.20	0.02
Cys	0.19	0.21	0.17	0.18	0.13	0.16	0.19	0.15	0.00
Glu	2.11	2.37	2.58	2.19	1.81	1.99	2.38	1.80	0.03
Gly	0.88	1.02	0.71	1.33	0.93	0.70	0.78	0.67	0.01
Pro	0.69	0.83	0.61	0.89	0.66	0.59	0.66	0.57	0.02
Ser	0.52	0.60	0.51	0.56	0.42	0.47	0.56	0.44	0.01
Tyr	0.59	0.67	0.68	0.59	0.51	0.62	0.70	0.57	0.02
Total	7.24	8.22	7.68	8.25	6.44	6.63	7.76	6.18	0.12
Total AA, %	14.61	16.43	15.67	15.89	12.66	13.71	16.13	12.84	0.25

[1]The nutrient composition of the N-free diet was analyzed.

[2]Diets were formulated to contain approximately 16% crude protein on an as-fed basis.

Table 4.5. Apparent ileal digestibility (AID) of amino acids (AA) in ingredients[1]

Item	Ingredient								Pooled SEM	*P*-value
	Salami	Bologna	Beef jerky	Ground beef		Ribeye roast				
				Raw	Cooked	Medium-rare	Medium	Well-done		
Indispensable AA, %										
Arg	93.4^{ab}	94.6^{a}	93.6^{ab}	94.3^{ab}	93.3^{b}	94.0^{ab}	93.6^{ab}	93.5^{ab}	0.50	0.367
His	90.5^{cd}	92.8^{ab}	91.8^{bc}	93.8^{a}	90.1^{d}	92.2^{b}	91.4^{bcd}	90.6^{cd}	0.59	<0.001
Ile	90.8^{cd}	92.2^{abc}	92.5^{ab}	92.9^{a}	90.6^{d}	92.1^{abc}	91.9^{abcd}	91.4^{bcd}	0.57	0.033
Leu	91.3^{bc}	92.1^{bc}	92.6^{ab}	93.5^{a}	91.0^{c}	92.4^{ab}	91.6^{bc}	91.6^{bc}	0.53	0.010
Lys	91.7^{bc}	92.7^{ab}	92.4^{b}	94.1^{a}	90.9^{c}	93.2^{ab}	92.2^{bc}	91.8^{bc}	0.59	0.004
Met	93.3^{c}	93.9^{bc}	94.0^{bc}	95.1^{a}	93.7^{bc}	95.1^{a}	95.1^{a}	94.5^{ab}	0.44	0.007
Phe	89.6^{bc}	90.7^{b}	91.0^{ab}	92.3^{a}	89.2^{c}	90.8^{ab}	90.0^{bc}	89.9^{bc}	0.59	0.008
Thr	85.6^{bc}	85.6^{bc}	86.5^{ab}	88.5^{a}	84.0^{c}	85.7^{bc}	84.9^{bc}	84.8^{bc}	0.86	0.017
Trp	89.0^{bc}	92.4^{a}	90.6^{ab}	89.3^{bc}	89.0^{bc}	89.6^{bc}	87.9^{c}	89.4^{bc}	1.00	0.034
Val	88.5^{ab}	89.1^{ab}	89.5^{ab}	90.2^{a}	87.7^{b}	89.1^{ab}	88.2^{b}	88.4^{ab}	0.76	0.266

Table 4.5 (cont.)

Mean	90.6^{bc}	91.7^{ab}	91.5^{bc}	93.1^{a}	90.2^{c}	91.7^{ab}	90.8^{bc}	90.8^{bc}	0.57	0.011
Dispensable AA, %										
Ala	89.8^{b}	91.1^{ab}	90.9^{b}	92.5^{a}	90.2^{b}	90.5^{b}	90.2^{b}	89.6^{b}	0.60	0.023
Asp	87.9^{bcd}	89.6^{ab}	88.6^{abc}	90.5^{a}	85.4^{e}	86.9^{cde}	86.0^{de}	83.3^{f}	0.85	$<$0.001
Cys	76.2^{abc}	77.7^{ab}	74.1^{bcd}	80.5^{a}	67.0^{e}	72.2^{cd}	73.1^{cd}	71.0^{de}	1.64	$<$0.001
Glu	91.6^{ab}	92.6^{a}	92.6^{a}	92.8^{a}	90.7^{b}	92.0^{ab}	91.8^{ab}	90.6^{b}	0.58	0.018
Gly	81.4^{bc}	87.8^{a}	79.7^{bc}	87.9^{a}	83.1^{b}	79.4^{c}	78.5^{c}	79.5^{bc}	1.50	$<$0.001
Ser	86.5^{abc}	87.8^{ab}	86.2^{abc}	88.7^{a}	84.4^{c}	85.8^{bc}	84.9^{c}	84.9^{c}	0.99	0.017
Tyr	91.0^{d}	91.7^{cd}	92.8^{bc}	94.1^{a}	91.7^{cd}	93.3^{ab}	92.3^{bcd}	92.6^{bc}	0.49	$<$0.001
Mean	87.5^{abc}	90.0^{a}	88.2^{ab}	89.3^{ab}	85.8^{c}	87.1^{bc}	87.4^{abc}	85.3^{c}	1.00	0.004
Total AA, %	89.0^{ab}	90.8^{a}	90.2^{a}	90.9^{a}	87.9^{b}	89.5^{ab}	89.6^{ab}	88.2^{b}	0.75	0.012

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

[1]Data are least squares means of 8 observations per treatment except for beef jerky and raw ground beef that have 7 observations per treatment and for salami and medium ribeye roast that have 6 observations per treatment.

Table 4.6. Standardized ileal digestibility (SID) of amino acids (AA) in ingredients[1,2]

Item	Salami	Bologna	Beef jerky	Ground beef		Ribeye roast			Pooled SEM	*P*-value
				Raw	Cooked	Medium-rare	Medium	Well-done		
Indispensable AA, %										
Arg	105.2^{bc}	105.3^{bc}	105.1^{c}	104.5^{c}	106.4^{ab}	106.4^{ab}	104.3^{c}	106.8^{a}	0.50	0.001
His	95.3^{c}	96.9^{bc}	95.8^{c}	98.8^{a}	96.4^{bc}	97.3^{ab}	95.9^{c}	96.3^{bc}	0.59	0.001
Ile	95.6^{b}	96.5^{ab}	96.7^{ab}	97.6^{a}	96.3^{ab}	97.0^{ab}	96.0^{b}	96.6^{ab}	0.57	0.261
Leu	96.2^{c}	96.7^{bc}	97.2^{bc}	98.5^{a}	97.0^{bc}	97.7^{ab}	96.1^{c}	97.2^{bc}	0.53	0.017
Lys	96.3^{bc}	96.8^{bc}	96.4^{bc}	98.4^{a}	96.2^{c}	97.7^{ab}	96.1^{c}	96.7^{bc}	0.59	0.026
Met	96.1^{c}	96.4^{bc}	97.0^{abc}	97.7^{a}	96.6^{bc}	97.5^{ab}	96.6^{abc}	97.0^{abc}	0.47	0.138
Phe	95.4^{c}	96.1^{bc}	96.5^{abc}	98.0^{a}	96.2^{bc}	97.0^{ab}	95.3^{c}	96.5^{abc}	0.59	0.028
Thr	96.0^{bc}	94.9^{bc}	96.2^{bc}	98.7^{a}	96.6^{ab}	96.7^{ab}	94.1^{c}	96.5^{ab}	0.86	0.017
Trp	96.2^{ab}	97.9^{a}	96.9^{ab}	98.2^{a}	97.6^{a}	97.1^{ab}	94.8^{b}	98.0^{a}	1.00	0.132
Val	95.7^{ab}	95.7^{ab}	96.4^{ab}	97.4^{a}	96.5^{ab}	96.9^{a}	94.7^{b}	96.5^{ab}	0.76	0.217
Mean	97.1^{bc}	97.5^{bc}	97.6^{bc}	99.4^{a}	97.9^{bc}	98.5^{ab}	96.6^{c}	98.0^{ab}	0.57	0.019

Table 4.6 (cont.)

Dispensable AA, %										
Ala	99.2^{ab}	99.7^{ab}	99.8^{ab}	100.3^{ab}	100.3^{ab}	100.8^{a}	98.9^{b}	100.4^{a}	0.60	0.221
Asp	95.1^{b}	96.1^{ab}	95.4^{ab}	97.5^{a}	94.1^{bc}	94.7^{bc}	92.6^{cd}	91.6^{d}	0.85	<0.001
Cys	91.0^{b}	90.7^{b}	89.9^{b}	96.0^{a}	88.3^{b}	89.7^{b}	87.8^{b}	89.2^{b}	1.64	0.016
Glu	96.9^{a}	97.4^{a}	97.0^{a}	98.0^{a}	96.9^{a}	97.7^{a}	96.6^{a}	96.9^{a}	0.58	0.573
Gly	109.1^{abc}	111.5^{ab}	111.6^{ab}	106.1^{c}	109.3^{abc}	114.1^{a}	107.9^{bc}	113.5^{a}	1.98	0.029
Ser	99.0^{ab}	98.6^{ab}	99.2^{ab}	100.3^{a}	99.8^{a}	99.8^{a}	96.6^{b}	99.7^{a}	0.99	0.140
Tyr	95.9^{d}	96.0^{d}	97.0^{bcd}	99.0^{a}	97.3^{bc}	98.0^{ab}	96.4^{cd}	97.6^{bc}	0.49	<0.001
Mean	110.0^{abc}	109.8^{abc}	109.4^{abc}	109.2^{bc}	111.1^{ab}	111.7^{a}	108.4^{c}	111.7^{a}	0.99	0.076
Total AA, %	103.5^{ab}	103.7^{ab}	103.7^{ab}	104.1^{ab}	104.6^{ab}	104.9^{a}	102.7^{b}	104.6^{ab}	0.75	0.359

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

[1]Data are least squares means of 8 observations per treatment except for beef jerky and raw ground beef that have 7 observations per treatment and for salami and medium ribeye roast that have 6 observations per treatment.

[2]Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses (g/kg of dry matter intake) AA were as follows: crude protein, 25.83; Arg, 1.16; His, 0.26;

Table 4.6 (cont.)

Ile, 0.35; Leu, 0.61; Lys, 0.58; Met, 0.09; Phe, 0.37; Thr, 0.69; Trp, 0.14; Val, 0.58; Ala, 0.84; Asp, 1.00; Cys, 0.28; Glu, 1.13; Gly, 2.43; Pro, 9.66; Ser, 0.65; Tyr, 0.29.

Table 4.7. Digestible indispensable amino acid scores (DIAAS) for the 8 meat ingredients[1]

Item	Salami	Bologna	Beef jerky	Ground beef		Ribeye roast			Pooled SEM	*P*-value
				Raw	Cooked	Medium-rare	Medium	Well-done		
DIAA reference ratio										
His	1.55	1.86	1.91	1.56	1.22	1.50	1.69	1.35		
Ile	1.37	1.53	1.57	1.42	1.15	1.34	1.60	1.27		
Leu	1.11	1.18	1.21	1.12	0.92	1.06	1.23	0.99		
Lys	1.32	1.47	1.51	1.43	1.16	1.36	1.58	1.26		
SAA[2]	1.07	1.20	1.02	1.30	0.97	1.13	1.38	1.08		
AAA[3]	1.39	1.54	1.56	1.45	1.20	1.41	1.60	1.31		
Thr	1.26	1.39	1.36	1.33	1.05	1.21	1.40	1.13		
Trp	1.35	1.78	1.56	1.11	1.15	1.31	1.38	1.16		
Val	1.12	1.21	1.18	1.13	0.93	1.04	1.21	0.99		
DIAAS, %										

Table 4.7 (cont.)

Child (6 m to 3 y)[4]	107[c] (SAA)	118[a] (Leu)	102[d] (SAA)	111[b] (Trp)	92[f] (Leu)	104[d] (Val)	121[a] (Val)	99[e] (Leu)	1.02	<0.001
DIAA reference ratio										
His	1.94	2.33	2.38	1.95	1.52	1.87	2.12	1.69		
Ile	1.47	1.63	1.68	1.51	1.22	1.43	1.71	1.36		
Leu	1.20	1.28	1.31	1.21	0.99	1.14	1.33	1.07		
Lys	1.57	1.75	1.79	1.69	1.37	1.62	1.87	1.49		
SAA[2]	1.26	1.41	1.20	1.52	1.14	1.33	1.62	1.27		
AAA[3]	1.76	1.95	1.98	1.84	1.53	1.78	2.02	1.67		
Thr	1.56	1.72	1.69	1.64	1.30	1.50	1.74	1.41		
Trp	1.73	2.29	2.00	1.43	1.48	1.69	1.78	1.50		
Val	1.20	1.30	1.26	1.21	1.00	1.11	1.30	1.07		
DIAAS, %										

Table 4.7 (cont.)

Older child, adolescent, adult[5]	120[b] (Val)	128[a] (Leu)	120[b] (SAA)	121[b] (Leu)	99[e] (Leu)	111[c] (Val)	130[a] (Val)	107[d] (Val)	0.97	<0.001

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

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

[2]SAA = sulfur amino acid.

[3]AAA = aromatic amino acid.

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

[5]DIAAS were calculated using the recommended AA scoring pattern for 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; sulfur AA, 23; aromatic AA, 41; Thr, 25; Trp, 6.6; Val, 40 (FAO, 2013).

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