Received: 5 June 2024 | Revised: 7 August 2024 | Accepted: 8 August 2024
DOI: 10.1002/jsf2.215

RESEARCH ARTICLE

JSFA Reports

Pork cuts, hams, and sausages have digestible indispensable amino acid scores (DIAAS) close to or over 100

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Abstract

Background: The "Digestible Indispensable Amino Acid Score" (DIAAS) method measures protein quality in human foods. Animal-based products generally have high protein quality, but DIAAS values for some pork products are not available. Therefore, the objective of this experiment was to determine standardized ileal digestibility (SID) of amino acids (AA) and DIAAS values for pork products, including three types of pork cuts, Italian hams, and sausages, and to test the hypothesis that all pork products have DIAAS equal to or greater than 100.

Results: The mean SID of the indispensable AA in all pork products was above 90% and indicated excellent digestibility. For children from 6 months to 3 years old and individuals older than 3 years, prosciutto had greater ($p < 0.05$) DIAAS than all other pork products. Tenderloin and Coppa had greater ($p < 0.05$) DIAAS than back ribs, shoulder butt, chorizo, and bratwurst. There were no limiting AA in any of the pork products (DIAAS >100) with the exception that sulfur AA (SAA: methionine + cysteine) were limiting in chorizo for children from 6 months to 3 years old (DIAAS = 99).

Conclusion: With the exception of chorizo for children less than 3 years old, all pork products have "excellent" protein quality for individuals older than 6 months of age, with DIAAS greater than 100. Pork proteins can, therefore, complement proteins with low quality to produce a meal that is adequate in all AA.

KEYWORDS
amino acids, digestibility, digestible indispensable amino acid score, pork, protein quality

INTRODUCTION

Insufficient intake of protein and amino acids (AA) by children results in protein malnutrition that is associated with stunted growth and impaired cognitive development as well as reduced bone marrow function.[1,2] Protein quality can be accessed for human foods using the Food and Agriculture Organization (FAO)-recommended method called "Digestible Indispensable Amino Acid Score" (DIAAS), which is based on the digestibility of each AA at the end of the small intestine (the ileum) of growing pigs as a model when humans are unavailable.[3] The DIAAS method may be applied to all types of foods and captures the effects of technological processing, and values for DIAAS measured in individual ingredients are additive in combined meals containing different foods.[3,4] Animal proteins usually have greater protein quality than plant proteins, with most animal proteins having DIAAS close to or greater than 100, indicating that the high-quality protein contained in animal-based products has the potential to complement lower-quality proteins.[5–7]

Pork is one of the most produced meats in the world,[8] and DIAAS in pork belly, bacon, bologna, burger (ground pork), ham (traditional), loin roast, and salami is greater than 100, demonstrating that these foods contain high-quality proteins.[4,9,10] Protein quality is expected to be high in all pork products, but because DIAAS for several pork products has not yet been reported, it is not possible to determine the AA adequacy of meals containing these foods. As an example, pork products such as some specific cuts, hams, and sausages are commonly consumed, but no DIAAS values have been reported for these products. Therefore, assessing the protein quality of meals containing these foods will provide not only the AA composition, but also the digestibility of each indispensable AA, and thereby enhance the ability of food professionals to prepare meals that are adequate in all indispensable AA. Therefore, the objective of this experiment was to determine the standardized ileal digestibility (SID) and DIAAS values for nine pork products when fed to growing pigs, and to test the hypothesis that all pork products have high protein quality with DIAAS equal to or greater than 100.

MATERIALS AND METHODS

The protocol for the animal experiment was submitted to and approved by the Institutional Animal Care and Use Committee at the University of Illinois, Urbana-Champaign, IL, USA (IACUC #21244). Female pigs that were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used.

Ingredients and experimental diets

Three types of pork cuts (i.e., back ribs, tenderloin, and shoulder butt), three types of Italian hams (i.e., Coppa, prosciutto, and speck), and three types of sausages (i.e., chorizo, Italian, and bratwurst) were used (Table 1). All pork products were purchased from commercial brands available in grocery stores. The back ribs (Gordon Choice®, Champaign, IL, USA), tenderloin (Halperns'®, Atlanta, GA, USA), and shoulder butt (Halperns'®) were whole roasted in a pre-heated convection oven (Ninja DT200 Series, FOODI™ XL Pro Air Oven, SharkNinja, Needham, MA, USA) at 177°C, 218°C, and 177°C, respectively, as recommended.[11] All pork cuts were cooked to a minimum internal temperature of 63°C following guidelines of time and preparation from the Food Safety and Inspection Service.[11] The Italian hams were purchased from Hormel Foods (Austin, MN, USA), Carando Foods® (Springfield, MA, USA), and Halperns'®. Sausages were purchased fully cooked from Primo Gusto® (distributed by Gordon Choice®) and Johnsonville Sausage Co®., Sheboygan Falls, WI, USA. According to information on the package, Coppa was made from smoked and cured

TABLE 1 Analyzed nutrient composition of pork products (as-fed basis).

	Pork cuts			Italian hams			Sausages		
Item, %	Back ribs	Shoulder butt	Tenderloin	Coppa	Prosciutto	Speck	Chorizo	Italian	Bratwurst
Dry matter	45.21	38.45	33.12	33.80	44.51	57.67	51.53	45.48	49.75
Crude protein	22.67	24.89	29.46	19.09	25.95	34.07	14.43	13.08	10.72
Indispensable AA									
Arginine	1.42	1.53	1.86	1.26	1.74	2.26	0.90	0.93	0.67
Histidine	0.81	0.78	1.12	0.69	1.06	1.02	0.47	0.48	0.36
Isoleucine	1.04	1.15	1.62	1.01	1.48	1.57	0.66	0.63	0.50
Leucine	1.67	1.85	2.44	1.59	2.24	2.58	1.05	1.02	0.80
Lysine	1.85	2.04	2.71	1.75	2.49	2.85	1.13	1.11	0.85
Methionine	0.57	0.62	0.83	0.46	0.65	0.76	0.27	0.28	0.19
Phenylalanine	0.85	0.94	1.25	0.82	1.15	1.33	0.55	0.53	0.42
Threonine	0.93	1.03	1.31	0.85	1.23	1.42	0.58	0.57	0.43
Tryptophan	0.28	0.34	0.45	0.26	0.39	0.44	0.17	0.17	0.12
Valine	1.10	1.18	1.61	1.05	1.50	1.69	0.70	0.70	0.52
Dispensable AA									
Alanine	1.26	1.35	1.66	1.20	1.63	2.04	0.80	0.79	0.61
Aspartic acid	1.95	2.13	2.81	1.87	2.60	3.00	1.23	1.17	0.91
Cysteine	0.24	0.26	0.33	0.24	0.31	0.36	0.16	0.15	0.11
Glutamic acid	2.97	3.36	4.52	2.99	4.20	4.84	1.67	1.66	1.41
Glycine	1.24	1.24	1.24	1.16	1.41	2.20	0.88	0.88	0.68
Proline	0.90	0.96	1.04	0.88	1.10	1.55	0.71	0.62	0.54
Serine	0.76	0.83	0.94	0.65	0.93	1.18	0.49	0.47	0.37
Tyrosine	0.58	0.64	0.78	0.40	0.65	1.07	0.53	0.42	0.41

Abbreviation: AA, amino acids.

TABLE 2 Ingredient composition of experimental diets (as-fed basis).[a]

	Pork cuts			Italian hams			Sausages			
Item, %	Back ribs	Shoulder butt	Tenderloin	Coppa	Prosciutto	Speck	Chorizo	Italian	Bratwurst	Nitrogen-free
Pork product	33.80	31.00	26.50	37.80	31.00	26.50	52.50	52.50	65.00	-
Corn starch	44.10	46.90	51.40	40.10	46.90	51.40	25.40	25.40	12.90	77.55
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Canola oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Solka floc	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	2.10
Limestone	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.55
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Potassium carbonate	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Magnesium dioxide	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[b]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[a]All diets, except the nitrogen-free diet, were formulated to contain approximately 10% crude protein (dryatter basis).

[b]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, 2208 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.

trimmed pork shoulder, prosciutto was made from cured non-smoked hind pork leg, and speck was made from a 6-month-cured lightly smoked hind pork leg (from prosciutto ham). Chorizo was made from cured ground pork, Italian sausage from cured lean pork, and bratwurst from fresh ground pork.

Nine experimental diets were prepared following published guidelines for the DIAAS method.[1] Each diet contained one pork product as the only source of crude protein (CP) and AA (Tables 2 and 3). A nitrogen-free diet was also prepared to enable the calculation of SID of CP and AA. All pork products were served with a premixed mixture of nitrogen-free ingredients including corn starch, sucrose, canola oil, and cellulose to provide a meal that contained 10% CP on a dry matter basis.[1] Vitamins and minerals were included in all diets to meet or exceed current requirement estimates for growing pigs.[12] Diets also contained 0.4% titanium dioxide as an indigestible marker. A sample of each ingredient and each diet was collected during diet mixing and used for chemical analysis.

Animal work

Nine growing female pigs with an initial average body weight of 50.3 ± 3.8 kg were used. Pigs had a T-cannula installed in the distal ileum when they had a body weight of approximately 30 kg following published procedures,[13] and they had been used in a previous experiment before being used in the current experiment. Female pigs are usually used in DIAAS experiments because most male pigs on commercial and research farms are castrated, and the use of female pigs, therefore, is believed to be an appropriate model for humans as long as the experiment is completed before animals reach puberty.[14] Pigs were fed a common grower diet for 1 week after the conclusion of the previous experiment before the start of this experiment. They were housed in individual pens (1.5 × 2.5 m) with smooth sides and half-slatted concrete floors in an environmentally controlled room. A feeder and a nipple drinker were installed in each pen. Pigs were randomly allotted to a 9 × 6 Youden Square design with 9 diets that each contained one of the pork products and six 7-day periods. Diets were changed among pigs in subsequent periods, and no pig received the same diet more than once during the experiment. There were, therefore, six replicate pigs per treatment. At least five replicate pigs must be used to obtain results representative of a food ingredient.[1] The nitrogen-free diet was fed to all pigs in one additional period because endogenous excretions were corrected on a pig basis rather than a period basis.[14]

All pork products were prepared in the form humans would usually consume them, but they were processed into smaller pieces (1–2 cm) with a food processor (4-Quart Food Processor with LiquiLock Seal System, WFP16SCND; Waring Commercial, Torrington, CT, USA) before being mixed with the protein-free premixes to allow proper homogenization with the marker.[1] All pigs were fed their assigned diets in a daily amount equivalent to 8% of body weight$^{0.75}$, calculated on a dry-matter basis. Daily feed allowances were divided into two equal meals that were provided at 0700 and 1600 h. Individual pig weights were recorded at the beginning of the experiment and at the conclusion of each period to calculate feed allowance for the following experimental period.

Each experimental period lasted 7 days, with the initial 5 days being the adaptation period to the diets whereas ileal digesta were collected for 9 h on days 6 and 7 using standard procedures.[13,14]

TABLE 3 Analyzed nutrient composition of experimental diets (as-fed basis).

	Pork cuts			Italian hams			Sausages			
Item, %	Back ribs	Shoulder butt	Tenderloin	Coppa	Prosciutto	Speck	Chorizo	Italian	Bratwurst	Nitrogen-free
Dry matter	76.46	75.10	77.30	70.85	78.18	82.88	71.27	68.21	65.53	91.20
Crude protein	7.41	7.04	7.28	7.20	7.94	9.12	7.56	7.32	6.38	0.26
Titanium[a]	0.23	0.22	0.24	0.24	0.23	0.24	0.24	0.22	0.22	0.22
Indispensable AA										
Arginine	0.46	0.38	0.39	0.45	0.48	0.75	0.40	0.44	0.43	0.01
Histidine	0.30	0.24	0.29	0.28	0.34	0.37	0.25	0.27	0.26	0.01
Isoleucine	0.38	0.32	0.37	0.38	0.45	0.59	0.32	0.35	0.32	0.01
Leucine	0.63	0.54	0.58	0.63	0.70	0.95	0.53	0.57	0.54	0.02
Lysine	0.70	0.59	0.63	0.69	0.77	1.03	0.56	0.63	0.58	0.02
Methionine	0.19	0.17	0.18	0.18	0.20	0.27	0.14	0.16	0.13	0.00
Phenylalanine	0.32	0.27	0.29	0.32	0.35	0.49	0.27	0.29	0.28	0.01
Threonine	0.34	0.29	0.31	0.33	0.38	0.51	0.28	0.31	0.29	0.01
Tryptophan	0.10	0.09	0.10	0.09	0.13	0.16	0.09	0.09	0.08	0.02
Valine	0.41	0.34	0.38	0.40	0.46	0.61	0.35	0.38	0.35	0.01
Dispensable AA										
Alanine	0.47	0.41	0.40	0.49	0.52	0.75	0.41	0.45	0.42	0.01
Aspartic acid	0.73	0.62	0.66	0.74	0.81	1.09	0.63	0.68	0.62	0.01
Cysteine	0.08	0.08	0.08	0.09	0.09	0.13	0.08	0.08	0.08	0.00
Glutamic acid	1.21	1.04	1.10	1.25	1.33	1.77	0.99	1.07	1.09	0.01
Glycine	0.44	0.38	0.29	0.49	0.46	0.80	0.43	0.48	0.45	0.01
Proline	0.34	0.29	0.25	0.37	0.36	0.65	0.33	0.35	0.33	0.01
Serine	0.28	0.24	0.23	0.27	0.29	0.41	0.23	0.26	0.24	0.01
Tyrosine	0.16	0.12	0.14	0.14	0.17	0.40	0.14	0.15	0.16	0.01

Abbreviation: AA, amino acids.
[a]Indigestible marker 30 Values represent the amount of titanium recovered from the titanium dioxide that was included in the diet. Titanium dioxide contains approximately 60% titanium.

Collected bags were immediately frozen at – 20°C to prevent bacterial degradation of the AA in the digesta. At the end of the experiment, pigs had an average body weight of 85.2 ± 8.4 kg.

Chemical analysis

At the conclusion of the experiment, ileal digesta samples were thawed and homogenized for each animal, and a sub-sample was lyophilized and finely ground using a common coffee grinder. Diets were also lyophilized and ground, which enabled the collection of a uniform sample of each diet that could be used for analysis. Diets, ingredients, and ileal digesta samples were analyzed for dry matter (method 930.15).[15] Nitrogen was also determined in these samples (method 990.03)[15] using a LECO FP628 Nitrogen analyzer (LECO Corp., Saint Joseph, MI, USA), and CP was calculated as nitrogen × 6.25. Amino acids were analyzed in all samples on a Hitachi Amino Acid Analyzer (Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA, USA). Prior to analysis, samples were hydrolyzed with 6 *N* HCl for 24 h at 110°C [method 982.30 E(a)].[15] Methionine and cysteine were determined as methionine sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [method 982.30 E(b)].[15] Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C [method 982.30 E(c)].[15] Diet and ileal digesta samples were also analyzed for titanium.[16]

Calculations

The apparent ileal digestibility (AID), basal endogenous losses, and SID of CP and all AA in each diet were calculated according to published equations.[17] Subsequently, the digestible indispensable AA reference ratio was calculated for each pork product using the following equation[3]:

$$\text{Reference ratio} = \frac{\text{Digestible indispensable AA content in 1 g protein (mg)}}{\text{Same dietary indispensable AA in 1 g of reference protein (mg)}}$$

The digestible indispensable AA content in 1 g protein (mg) was calculated by multiplying each AA concentration by its respective SID and dividing this quantity by the protein content in each pork product. The dietary indispensable AA in 1 g of reference protein (mg) was derived from published values for children from 6 months to 3 years and for individuals older than 3 years.[3]

The DIAAS values were then calculated using the following equation[3]:

$$\text{DIAAS}\,(\%) = 100 \times \text{Lowest value of the reference ratio}$$

Statistical analysis

Data were analyzed by ANOVA using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA) with pig as the experimental unit. The normality of residuals and homogeneity of variances were confirmed using the UNIVARIATE procedure. Brown and Forsythe's test was also used to check for variance homogeneity. When this assumption was not met, data were transformed using the BOXCOX procedure, and assumptions were re-checked. Outliers were detected as observations that deviated from the treatment mean by ±3 times the interquartile range. The statistical model included diet as the fixed effect and pig and period as random effects. Treatment means were calculated using the LSMEANS statement in SAS, and if significant, means were separated using the PDIFF option in the MIXED procedure with Tukey's adjustment to control for type-1 experiment-wise error. An alpha value of 0.05 was used to assess significance among means.

RESULTS

All pigs remained healthy throughout the experiment and readily consumed their diets.

Amino acid digestibility

The AID of CP was greater ($p < 0.05$) in speck and chorizo compared with tenderloin and Coppa (Table 4). With a few exceptions, speck had a greater ($p < 0.05$) AID of all AA compared with all other pork products except prosciutto and bratwurst, and with a few exceptions, tenderloin had the least ($p < 0.05$) AID of AA.

The SID of CP, histidine, lysine, and glycine did not significantly differ among pork products (Table 5). For most of the other AA, bratwurst had greater ($p < 0.05$) SID than tenderloin, Coppa, and chorizo, and tenderloin had the least ($p < 0.05$) SID compared with all the other pork products. However, regardless of product, all indispensable AA had an average SID value greater than 90%.

DIAAS

For both age groups, prosciutto had greater ($p < 0.05$) DIAAS compared with the other pork products (Table 6). Tenderloin and Coppa had greater ($p < 0.05$) DIAAS than back ribs, shoulder butt, chorizo, and bratwurst, and Coppa also had greater ($p < 0.05$) DIAAS than tenderloin for individuals older than 3 years of age. For children between 6 months and 3 years of age, bratwurst and chorizo had a DIAAS that was less ($p < 0.05$) than for all other pork products, Italian sausage had a DIAAS that was greater ($p < 0.05$) than the DIAAS for back ribs and shoulder butt, and speck had a DIAAS that was greater ($p < 0.05$) than that for back ribs. For individuals older than 3 years, Italian sausage had a DIAAS that was greater ($p < 0.05$) than the DIAAS for back ribs, shoulder butt, chorizo, bratwurst and speck, and speck had a DIAAS that was greater ($p < 0.05$) than the DIAAS for back ribs and shoulder butts, whereas bratwurst had a DIAAS that was greater ($p < 0.05$) than that for chorizo.

For both ages, there was no limiting AA for any pork product (DIAAS >100), with the exception that for children from 6 months to 3 years old, chorizo had sulfur amino acids (SAA: methionine + cysteine) as the first limiting AA (DIAAS = 99). For children from 6 months to 3 years old, minimum and maximum values for DIAAS greater than 100 were 101 (bratwurst) and 127 (prosciutto), but for individuals older than 3 years, DIAAS values were between 113 (chorizo) and 137 (prosciutto).

DISCUSSION

Diet analysis indicated that the intended concentrations of CP and AA were present in all experimental diets. Overall, the analyzed nutrient composition of all pork products was in agreement with published values for these food ingredients.[9,18]

The SID of AA in all pork products was in agreement with published values for other pork products and meats,[4,6,9] with values above 90% for almost all AA, indicating high digestibility, which means that almost all indispensable AA in these products are absorbed and made available for body protein synthesis.[17] Values for SID are corrected for basal endogenous losses, and the reason some values were above 100% is that the use of a nitrogen-free diet to estimate basal endogenous losses of AA sometimes results in an overestimation of

TABLE 4 Apparent ileal digestibility (AID) of crude protein (CP) and amino acids (AA) in pork products.[†]

	Pork cuts			Italian hams			Sausages				
Item, %	Back ribs	Shoulder butt	Tenderloin	Coppa	Prosciutto	Speck	Chorizo	Italian	Bratwurst	SEM[‡]	*p*-value
Crude protein	68.9^{ab}	67.7^{ab}	60.6^{c}	66.3^{bc}	68.2^{ab}	72.9^{a}	72.8^{a}	70.9^{ab}	70.5^{ab}	3.52	0.009
Indispensable AA											
Arginine	79.6^{bcd}	72.8^{e}	66.0^{f}	77.9^{cde}	75.9^{de}	86.2^{a}	84.0^{abc}	81.6^{abcd}	84.5^{ab}	3.55	<0.001
Histidine	89.5^{ab}	88.8^{abc}	87.3^{c}	88.1^{bc}	90.2^{a}	90.3^{a}	88.2^{bc}	89.6^{ab}	90.6^{a}	0.81	0.015
Isoleucine	86.1^{de}	86.5^{cde}	83.5^{f}	86.5^{de}	89.3^{ab}	90.4^{a}	84.3^{ef}	87.4^{bcd}	88.8^{abc}	0.95	<0.001
Leucine	87.1^{cd}	87.4^{bcd}	84.0^{e}	87.1^{cd}	89.4^{ab}	91.0^{a}	85.3^{de}	87.8^{bc}	89.1^{abc}	0.93	<0.001
Lysine	85.1^{bcd}	85.5^{bcd}	81.7^{d}	83.7^{cd}	87.7^{ab}	90.3^{a}	83.8^{bcd}	87.4^{abc}	84.1^{bcd}	1.53	0.007
Methionine	91.4^{bc}	92.3^{ab}	89.9^{c}	91.2^{bc}	93.4^{a}	93.3^{a}	90.0^{c}	91.9^{ab}	92.9^{ab}	0.71	0.002
Phenylalanine	84.7^{cd}	84.9^{bcd}	80.5^{e}	84.7^{cd}	86.9^{bc}	89.4^{a}	82.7^{de}	85.7^{bc}	87.4^{ab}	1.08	<0.001
Threonine	78.0^{b}	77.6^{b}	72.4^{c}	76.4^{bc}	79.4^{b}	84.9^{a}	72.7^{c}	78.3^{b}	80.2^{b}	1.64	<0.001
Tryptophan	82.5^{de}	83.9^{cd}	77.1^{f}	80.2^{ef}	87.5^{ab}	88.8^{a}	83.9^{bcd}	85.3^{abcd}	87.3^{abc}	1.53	<0.001
Valine	83.6^{e}	84.5^{cde}	79.6^{f}	84.1^{de}	86.9^{abc}	88.8^{a}	82.1^{ef}	86.1^{bcd}	87.3^{ab}	1.21	<0.001
Dispensable AA											
Alanine	80.0^{bc}	78.0^{c}	70.7^{d}	80.9^{bc}	80.7^{bc}	85.4^{a}	80.4^{bc}	82.0^{abc}	83.6^{ab}	2.17	<0.001
Aspartic acid	82.9^{bc}	82.6^{bcd}	78.0^{e}	82.4^{cd}	83.9^{bc}	88.1^{a}	80.0^{de}	84.0^{bc}	85.3^{ab}	1.20	<0.001
Cysteine	55.3^{cde}	59.9^{bcd}	49.8^{e}	60.0^{bcd}	62.7^{bc}	75.0^{a}	53.2^{de}	63.2^{bc}	65.7^{b}	3.14	0.0001
Glutamic acid	87.4^{b}	87.5^{b}	84.1^{c}	87.5^{b}	88.8^{ab}	90.7^{a}	85.1^{c}	87.7^{b}	89.8^{a}	0.91	<0.001
Glycine	53.7^{bc}	42.0^{c}	15.3^{d}	61.3^{ab}	49.4^{bc}	74.2^{a}	63.5^{ab}	55.6^{bc}	61.1^{ab}	7.40	<0.001
Serine	75.7^{bc}	73.8^{c}	66.9^{d}	73.7^{c}	74.8^{bc}	83.4^{a}	71.9^{c}	76.3^{bc}	78.6^{b}	1.94	<0.001
Tyrosine	78.0^{cde}	73.2^{fg}	70.1^{g}	76.1^{ef}	81.2^{c}	91.3^{a}	76.9^{def}	80.1^{cd}	86.9^{b}	1.72	<0.001

Note: $^{a-g}$Means within a row lacking a common superscript letter differ ($p < 0.05$).
[†]Data are means of six observations per treatment, except for the shoulder butt, speck, and chorizo that had five observations per treatment.
[‡]SEM, standard error of the mean. SEM and *p*-values were calculated based on one-way ANOVA of the mean values for each variable (CP or AA) for AID across the nine treatments.

the endogenous losses of arginine and glycine.[19] Nevertheless, using a nitrogen-free diet is preferred over other methods because of its simplicity, and results are usually in agreement with regression methods.[1,17]

Pork cuts are usually cooked before consumption because they can potentially carry harmful pathogens such as bacteria, parasites, and viruses that can cause foodborne illnesses if consumed raw or undercooked.[20] However, cooking may negatively affect protein quality because of modification of some indispensable AA, and cooking at increased temperatures may reduce AA digestibility and, as a consequence, DIAAS.[9] Nevertheless, results of this experiment indicated that whole roasting tenderloin, back ribs, and shoulder butt did not seem to have a negative impact on protein quality because values for DIAAS for both age groups were greater than 100. In addition, the lysine concentration in the cooked pork cuts was not reduced when compared with the lysine content in the same raw pork cuts,[18] and the AID lysine was also not reduced when compared with AID threonine.[4] This indicates that overheating may not have occurred because lysine is the first AA to be affected when overheating occurs due to the Maillard reaction, which can lead to a reduction in lysine concentration and digestibility.[21] However, additional research on reactive lysine would be required to confirm this hypothesis.

A food item can be considered to have a “good” or “excellent” quality of protein if DIAAS is between 75 and 99 or 100 and greater, respectively, but no protein claim can be made for a food with a DIAAS below 75.[3] The results for DIAAS for back ribs, shoulder butt, and tenderloin were in agreement with published values for raw belly, smoked bacon, and pork loin, respectively,[9] with DIAAS greater than 100 indicating “excellent” protein quality. The observation that back ribs and shoulder butt had lower DIAAS than tenderloin is likely a result of tender cuts, such as tenderloin, having less connective tissues and greater concentrations of indispensable AA with less non-protein nitrogen content compared with other muscle meats,[22] which results in increased DIAAS. It is possible that if back ribs and shoulder butt were slow-cooked, protein quality would be comparable to the tenderloin because when the connective tissue is simmered at low temperatures, it becomes more tender, possibly increasing the digestibility of AA and thereby contributing to an improvement in DIAAS, but further research to demonstrate this assumption is needed.

The calculated DIAAS for Coppa and prosciutto was, in general, within the DIAAS range of 115 to 133, which has been reported for cured ham.[9] The observation that prosciutto had a DIAAS greater than Coppa is likely a result of prosciutto being made from the hind leg of the pig, which likely contains less fat and collagen than pork

TABLE 5 Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in pork products.†,‡

	Pork cuts			Italian hams			Sausages				
Item, %	Back ribs	Shoulder butt	Tenderloin	Coppa	Prosciutto	Speck	Chorizo	Italian	Bratwurst	Sem§	*p*-value
Crude protein	93.7	93.4	86.3	89.8	91.7	94.6	95.4	93.3	95.0	3.31	0.094
Indispensable AA											
Arginine	98.7^{abc}	95.8^{bc}	89.3^{d}	96.0^{bc}	94.4^{cd}	98.8^{abc}	104.7^{a}	99.7^{abc}	102.1^{ab}	3.17	0.002
Histidine	96.6	97.4	94.8	95.1	96.3	96.3	96.0	96.5	97.5	0.80	0.103
Isoleucine	94.9^{bcde}	96.8^{abc}	92.8^{e}	94.5^{cde}	96.9^{ab}	96.5^{abcd}	94.1^{de}	95.8^{abcd}	97.6^{a}	0.93	0.004
Leucine	95.4^{abd}	97.0^{ab}	93.3^{d}	94.7^{bd}	96.8^{ac}	96.9^{ab}	94.6^{bcd}	95.8^{ab}	97.3^{a}	0.90	0.011
Lysine	94.8	96.9	92.8	92.5	96.2	97.2	95.1	96.4	93.6	1.49	0.130
Methionine	96.0^{bc}	97.6^{ab}	95.0^{c}	95.7^{c}	97.7^{ab}	96.8^{abc}	95.7^{c}	96.5^{bc}	98.5^{a}	0.69	0.005
Phenylalanine	94.4^{abc}	96.3^{ab}	91.7^{d}	93.6^{bcd}	95.8^{abc}	96.2^{abc}	93.4^{cd}	95.0^{abc}	96.9^{a}	1.04	0.007
Threonine	93.7^{ab}	95.6^{a}	90.2^{b}	91.3^{b}	93.9^{ab}	96.4^{a}	90.8^{b}	93.8^{ab}	96.1^{a}	1.66	0.030
Tryptophan	92.3^{bc}	94.6^{abc}	87.0^{d}	91.0^{c}	95.0^{ab}	95.7^{ab}	93.6^{bc}	94.8^{ab}	97.7^{a}	1.71	<0.001
Valine	93.9^{bc}	96.5^{ab}	90.8^{d}	93.5^{cd}	95.9^{abc}	96.1^{abc}	93.1^{cd}	95.6^{abc}	97.2^{a}	1.19	0.001
Dispensable AA											
Alanine	96.3^{a}	96.8^{a}	90.4^{b}	95.4^{a}	95.8^{a}	96.5^{a}	98.4^{a}	97.3^{a}	99.3^{a}	2.03	0.016
Aspartic acid	94.0^{abc}	95.4^{ab}	90.7^{d}	92.5^{bcd}	94.2^{abc}	96.2^{a}	92.0^{cd}	94.5^{abc}	96.4^{a}	1.19	0.003
Cysteine	80.0^{bc}	87.0^{ab}	77.1^{c}	80.7^{bc}	84.8^{abc}	91.7^{a}	77.5^{c}	85.2^{abc}	87.7^{ab}	3.20	0.025
Glutamic acid	95.5^{abc}	96.8^{ab}	93.2^{d}	94.7^{bcd}	96.3^{abc}	96.7^{ab}	94.3^{cd}	95.7^{abc}	97.5^{a}	0.89	0.008
Glycine	94.1	87.8	79.0	94.1	88.2	98.1	101.1	88.9	94.9	6.65	0.111
Serine	95.2^{ab}	95.8^{ab}	90.7^{c}	92.2^{bc}	93.7^{abc}	97.7^{a}	94.1^{abc}	94.7^{abc}	97.9^{a}	1.98	0.049
Tyrosine	93.3^{c}	93.4^{c}	88.2^{d}	91.8^{cd}	95.4^{bc}	97.9^{ab}	93.6^{bc}	94.4^{bc}	99.5^{a}	1.66	0.0003

Note: $^{a-e}$Means within a row lacking a common superscript letter differ ($p < 0.05$).
†Data are means of six observations per treatment, except for the shoulder butt, speck, and chorizo that had five observations per treatment.
‡The SID values were calculated by correcting values for basal ileal endogenous losses for each pig as its own control.
§SEM, standard error of the mean. SEM and *p*-values were calculated based on one-way ANOVA of the mean values for each variable (CP or AA) for SID across the nine treatments.

shoulder that is used to make Coppa,[23] which may have contributed to greater concentrations of indispensable AA. Speck was made from lightly smoked prosciutto ham after a 6-month curing process and had the lowest DIAAS among all Italian hams. It is possible that curing for an extended period of time may have reduced protein quality due to the formation of biogenic amines during ham ripening.[24] Nevertheless, DIAAS for this pork product was above 100%, demonstrating that speck, like the other hams, has excellent protein value. Curing with nitrite or nitrate and sodium chloride may also reduce protein oxidation, protecting the physical and chemical properties of proteins,[9] which may have contributed to the greater DIAAS in hams compared with the other pork products. Therefore, results for DIAAS for all Italian hams demonstrated that these proteins can be considered "excellent" protein quality.

To our knowledge, this is the first time DIAAS values for sausages are reported. All sausages were considered "good" or "excellent" quality proteins for individuals older than 6 months. The lower DIAAS for chorizo and bratwurst than Italian sausage is likely a result of processing or addition of fillers, binders, and spices that may dilute the AA content and lower the overall protein quality.[9,25] However, all sausages had DIAAS values above 100% for individuals older than 3 years, and for children from 6 months to 3 years, only chorizo with a DIAAS of 99 was below 100. All sausages were made from ground pork (along with water, salt, spices, and color additives), for which a digestibility of more than 95% for most indispensable AA and a DIAAS ranging from 111 to 119 have been reported.[4] These values are close to the values obtained in this experiment, which indicates that the cooking methods used to produce the sausages did not have a negative impact on AA digestibility.

Using ingredients that have DIAAS greater than 100 can improve the overall protein quality of a mixed meal. Usually, people consume meals with several foods, and high-quality proteins, such as animal proteins, can complement lower-quality proteins if sufficient quantities are consumed. As an example, for individuals older than 3 years, DIAAS ranged from 113 to 137 in the pork products used in this experiment, which is in agreement with DIAAS in other pork products, including raw pork belly, bacon, ham, and loin.[9] The DIAAS in beef and dairy products ranges from 99 to 130,[10] and 123 to 142,[7] respectively. In contrast, grains including corn, barley, oats, rice, rye, sorghum, and wheat, have a DIAAS between 29 and 77.[7,26,27] Therefore,

TABLE 6 Reference ratios and digestible indispensable amino acid score (DIAAS) for pork products.[†]

	Pork cuts			Italian hams			Sausages				
Item	Back ribs	Shoulder butt	Tenderloin	Coppa	Prosciutto	Speck	Chorizo	Italian	Bratwurst	SEM[3]	*p*-value
Child[‡]											
Reference ratio											
Histidine	1.72	1.53	1.81	1.71	1.97	1.44	1.56	1.76	1.63		
Isoleucine	1.37	1.39	1.59	1.56	1.72	1.39	1.34	1.44	1.42		
Leucine	1.06	1.09	1.17	1.19	1.27	1.11	1.04	1.13	1.09		
Lysine	1.36	1.39	1.50	1.49	1.62	1.43	1.31	1.44	1.30		
SAA	1.20	1.24	1.31	1.22	1.27	1.16	0.99	1.13	1.01		
AAA	1.15	1.16	1.19	1.15	1.27	1.31	1.35	1.32	1.46		
Threonine	1.24	1.28	1.30	1.31	1.43	1.30	1.17	1.32	1.24		
Tryptophan	1.34	1.51	1.57	1.46	1.69	1.45	1.30	1.43	1.28		
Valine	1.06	1.07	1.15	1.19	1.29	1.11	1.05	1.18	1.09		
DIAAS, %	106^{e} (Valine)	107^{de} (Valine)	115^{b} (Valine)	115^{bc} (AAA)	127^{a} (Leucine)	111^{cd} (Valine)	99^{f} (SAA)	113^{bc} (Leucine)	101^{f} (SAA)	1.58	<0.001
Older child, adolescent, adult[§]											
Reference ratio											
Histidine	2.16	1.92	2.26	2.14	2.47	1.80	1.95	2.20	2.04		
Isoleucine	1.46	1.48	1.70	1.67	1.84	1.48	1.43	1.53	1.51		
Leucine	1.15	1.18	1.27	1.29	1.37	1.20	1.13	1.22	1.18		
Lysine	1.61	1.65	1.78	1.77	1.92	1.69	1.56	1.70	1.55		
SAA	1.41	1.45	1.54	1.43	1.49	1.37	1.16	1.33	1.18		
AAA	1.45	1.47	1.51	1.46	1.61	1.66	1.71	1.67	1.86		
Threonine	1.54	1.59	1.61	1.62	1.78	1.61	1.45	1.63	1.53		
Tryptophan	1.73	1.95	2.02	1.88	2.17	1.87	1.67	1.85	1.65		
Valine	1.14	1.15	1.24	1.28	1.38	1.19	1.13	1.27	1.17		
DIAAS, %	114^{fg} (Valine)	115^{fg} (Valine)	124^{c} (Valine)	128^{b} (Valine)	137^{a} (Leucine)	119^{de} (Valine)	113^{g} (Leucine)	122^{cd} (Leucine)	117^{ef} (Valine)	1.26	<0.001

Note: $^{a-g}$Means within a row lacking a common superscript letter differ ($p < 0.05$).

[†]First-limiting AA is in parentheses. AAA, aromatic amino acids (phenylalanine + tyrosine); SAA, sulfur amino acids (methionine + cysteine); SEM, standard error of the mean.

[‡]DIAAS were calculated using the recommended AA scoring pattern for a child (6 months to 3 years). The indispensable AA reference patterns are expressed as mg AA/g protein: Histidine, 20; Isoleucine, 32; Leucine, 66; Lysine, 57; Sulfur AA, 27; Aromatic AA, 52; Threonine, 31; Tryptophan, 8.5; and Valine, 43.[3]

[§]DIAAS were calculated using the recommended AA scoring pattern for an older child, adolescent, and adult. The indispensable AA reference patterns are expressed as mg AA/g protein: Histidine, 16; Isoleucine, 30; Leucine, 61; Lysine, 48; Sulfur AA, 23; Aromatic AA, 41; Threonine, 25; Tryptophan, 6.6; and Valine, 40.[3]

combining pork products with plant proteins can produce meals with increased protein quality because they can be balanced in all indispensable AA. In addition, results from this experiment contribute to generating data for AA digestibility and protein quality of human foods that can be included in a human food database, which is needed to formulate diets with adequate protein quality. Because such data cannot be generated from humans, the growing pig is the preferred model for this work because AA digestibility in pigs is very similar to that in humans.[1]

CONCLUSION

Back ribs, shoulder butt, tenderloin, Coppa, prosciutto, speck, chorizo, Italian sausage, and bratwurst used in this experiment have high digestibility of indispensable AA, and DIAAS in these pork products indicated high protein quality for individuals older than 6 months of age, making them suitable for complementing low-quality proteins and providing a meal with balanced indispensable AA. However, chorizo had SAA as the first limiting AA for children from 6 months to

3 years old, but protein quality was considered "good" for this age, whereas all other proteins had "excellent" quality for children over 6 months and individuals older than 3 years. Whole-roasting pork cuts following recommended procedures from the Food Safety and Inspection Service did not negatively affect protein quality.

ACKNOWLEDGMENTS

Financial support for this study from Pork Checkoff (National Pork Board, Des Moines, IA) is greatly appreciated.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data from the experiment are presented in the manuscript.

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How to cite this article: Fanelli NS, Martins JCFR, Stein HH. Pork cuts, hams, and sausages have digestible indispensable amino acid scores (DIAAS) close to or over 100. JSFA Reports. 2024;4(9):326–35. https://doi.org/10.1002/jsf2.215