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Pork Products Have Digestible Indispensable Amino Acid Scores (DIAAS) That Are Greater Than 100 When Determined in Pigs, but Processing Does Not Always Increase DIAAS

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ABSTRACT

Background: Raw meat contains all indispensable amino acids (IAAs), but before human consumption, meat usually undergoes some degree of processing. Processing affects the 3-dimensional structure of proteins, which may affect amino acid (AA) digestibility and, therefore, overall protein quality.

Objectives: The experiment aimed at determining digestible indispensable amino acid scores (DIAAS) for pork products, and to test the hypothesis that processing increases DIAAS.

Methods: Ten ileal cannulated gilts (body weight: 26.63 ± 1.62 kg) were randomly allotted to a 10 × 10 Latin square design with ten 7-d periods. Ileal digesta were collected for 9 h on days 6 and 7 of each period. 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, and loins heated to 63°C, 68°C, or 72°C) as the sole source of AAs. A nitrogen-free diet was formulated to determine basal endogenous losses of AAs, which enabled calculation of standardized ileal digestibility (SID) of AAs. DIAAS were subsequently calculated according to the FAO.

Results: All pork products had DIAAS >100 (as-is basis). Loin heated to 63°C had the greatest ($P < 0.05$) DIAAS for children 6 mo to 3 y and smoked-cooked bacon had the greatest ($P < 0.05$) DIAAS for children older than 3 y, adolescents, and adults. Raw belly, smoked bacon, and loins heated to 68°C and 72°C had a reduced ($P < 0.05$) DIAAS for both reference patterns compared with other proteins. Alternatively cured ham had greater ($P < 0.05$) DIAAS when compared with non-cured ham and conventionally cured ham.

Conclusions: Bacon, ham, and loin are excellent proteins with DIAAS >100, and processing may sometimes, but not always, increase DIAAS. *J Nutr* 2020;150:475–482.

Keywords: amino acid, digestible indispensable amino acid score, protein quality, digestibility, pork, meat processing, pig model

Introduction

Pork is the most widely consumed animal meat protein in the world, accounting for >36% of global meat intake (1). Pork is also a balanced source of protein providing all essential amino acids (AAs) (2, 3). 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 (4). Meat is highly perishable, therefore processing almost always takes place before consumption to slow or inhibit microbial growth. Desirable sensory attributes are also developed with various forms of processing. Consequently, thermal processing induces modification of the 3-dimensional structure of the proteins, which may lead to increased digestibility of AAs (5, 6).

Protein quality can be evaluated in human foods using the digestible indispensable amino acid scores (DIAAS) determined by comparing the digestibility of individual dietary indispensable AAs (IAAs) in a food with the same IAA in the reference protein (7). The digestibility of each IAA is determined at the end of the small intestine (the ileum) and corrected for basal endogenous losses of AAs. Data for DIAAS obtained in humans are preferred, but if unavailable, the pig is recognized as a preferred model for determining DIAAS for human foods (7).

The effect of cooking on protein structure has been studied (5, 8, 9), and DIAAS have been determined for bovine meat cooked by various techniques and to a common internal temperature (10). However, despite the presumed high quality of pork protein, to our knowledge, there are no reported DIAAS for pork products and the IAA digestibility of pork

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TABLE 1 Cooking procedure of the 9 pork products fed to growing pigs

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

[1]Purchased in accordance with the Institutional Meat Purchasing Specifications (IMPS #413; 11, 12).
[2]Hams were classified as boneless fresh pork leg, inside (IMPS #402F; 11, 12).
[3]Loins were classified as fresh, boneless pork loin roast (IMPS #413; 11, 12).

after processing has not been reported. Therefore, the present experiment was conducted to test the hypothesis that pork proteins have DIAAS >100 and that processing increases DIAAS.

Methods

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

Preparation of pork products

Nine pork products were collected and prepared for DIAAS determination at the Meat Science Laboratory at North Dakota State University (**Tables 1** and **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 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°C (medium), 68°C (medium-well), and 72°C (well-done), respectively. The raw, primal cuts can be identified in The Meat Buyers Guide and Institutional Meat Purchasing Specifications (IMPS) sections for ham (IMPS #402F), pork belly (IMPS #408), and loin (IMPS #413; 11, 12). The smoked bacon and smoked-cooked bacon were processed in accordance with Appendix A of the Food Safety and Inspection Service Compliance Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products (13). The raw belly and all ham and loin products were coarse ground after processing, 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.) 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°C until use.

Animals, housing, diets, and feeding

Ten growing, heterozygous Yorkshire female pigs (initial body weight: 26.63 ± 1.62 kg) were surgically fitted with a T-cannula in the distal ileum as described by Stein et al. (14). 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. After a 7-d recovery period from surgery, pigs were allotted to a 10 × 10 balanced Latin square design with 10 diets and ten 7-d periods (15). The initial 5 d of each period was considered the adaptation phase to the diets, and ileal digesta were collected for 9 h on days 6 and 7 following procedures explained by Stein et al. (14). 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 source of crude protein (CP) and AAs (**Supplemental Tables 1** and **2**). These diets were prepared by homogeneously mixing an N-free mixture with the quantity of each pork product that was required to produce a final mix containing ∼16% CP. The N-free mixture was also fed without including any protein to determine basal endogenous losses of CP and AAs from the pigs, which was necessary for the calculation of DIAAS. 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 (16).

Feed was provided daily to each pig in an amount equivalent to 4% of body weight. Feed allowances were supplied in 2 equal daily meals at 08:00 and 17:00, and water was available at all times. Feed refusals, if any, were weighed daily, and all pigs were weighed at

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Address correspondence to HHS (e-mail: hstein@illinois.edu).
Abbreviations used: AA, amino acid; AID, apparent ileal digestibility; CP, crude protein; DIAAS, digestible indispensable amino acid scores; DM, dry matter; IAA, indispensable amino acid; IMPS, institutional meat purchasing specifications; SID, standardized ileal digestibility.

TABLE 2 Analyzed nutrient composition of the 9 pork products and the N-free diet (as-fed basis) that were fed to growing pigs[1]

	Raw belly	Bacon		Ham			Loin			N-free
		Smoked	Smoked-cooked	Non-cured	Alt. cured	Conv. cured	63°C	68°C	72°C	
GE, kcal/kg	3588	2835	4921	2143	1647	1543	1974	1949	2081	3699
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
AEE, %	31.77	19.34	27.58	3.42	2.67	2.27	5.34	5.44	4.67	1.38
Indispensable AAs										
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
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 AAs										
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
Total, %	7.74	8.87	19.85	16.62	12.07	11.24	13.61	14.06	15.49	0.16
Total AAs, %	15.61	17.92	38.93	34.71	25.09	23.33	28.45	29.23	31.85	0.31

[1]Values are means of 3 replicate analyses for GE, dry matter, crude protein, ash, and AEE, and values for all AAs are means of 2 replicate analyses. AA, amino acid; AEE, acid hydrolysis ether extract; alt. cured, alternatively cured ham; conv. cured, conventionally cured ham; GE, gross energy.

the beginning of each period to calculate feed allowance during the following period, and all pigs were weighed at the conclusion of the experiment.

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. Before 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 dry matter (DM; Method 930.15; 17) and AAs [Method 982.30 E (a, b, c); 17]. The N-free diet and ileal digesta samples were analyzed in duplicate for titanium (18), and CP was analyzed in those samples by the combustion procedure (Method 990.03; 17) using a LECO FP628 analyzer (LECO Corp.). All pork products were analyzed in triplicate for CP using the Kjeldahl method (Method 984.13; 17) on a Kjeltec™ 8400 (FOSS Inc.). Pork products and the N-free diet were analyzed in triplicate for ash at 600°C for 12 h (Method 942.05; 17), and for gross energy using an isoperibol bomb calorimeter (Model 6400; Parr Instruments) with benzoic acid as the standard for calibration. Acid hydrolyzed ether extract (AEE) was analyzed in the N-free diet and the pork products in triplicate using the acid hydrolysis filter bag technique (Ankom HCl Hydrolysis System, Ankom Technology) followed by crude fat extraction using petroleum ether (AnkomXT15 Extractor, Ankom Technology).

Calculations

Apparent ileal digestibility (AID) of CP and AAs in diets was calculated using Equation 1 (19).

$$\text{AID}\ (\%) = [1 - (\text{AA}_d/\text{AA}_f) \times (\text{Ti}_f/\text{Ti}_d)] \times 100, \tag{1}$$

where AID is the apparent ileal digestibility of an AA (%), AA_d is the concentration of that AA in the ileal digesta DM, AA_f is the calculated AA concentration of that AA in the diet DM, Ti_f is the calculated titanium concentration in the diet DM, and 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 (19).

$$\text{IAA}_{end} = [\text{AA}_d \times (\text{Ti}_f/\text{Ti}_d)], \tag{2}$$

where IAA_{end} is the basal endogenous loss of an AA (mg/kg dry matter intake). The basal endogenous loss of CP was determined using the sam e 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 (19).

$$\text{SID}\ (\%) = \text{AID} + [(\text{IAA}_{end}/\text{AA}_f) \times 100], \tag{3}$$

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

TABLE 3 AID of AAs in the 9 pork products fed to growing pigs[1]

		Bacon		Ham			Loin				
	Raw belly	Smoked	Smoked-cooked	Non-cured	Alt. cured	Conv. cured	63°C	68°C	72°C	SEM	*P* value
n[2]	8	9	10	9	9	9	9	8	8		
Indispensable AAs											
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
Dispensable AAs											
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 AAs, %	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

[1] Values are means and pooled SEMs. Labeled means in a row without a common superscript letter differ, $P < 0.05$. AA, amino acid; AID, apparent ileal digestibility; alt. cured, alternatively cured ham; conv. cured, conventionally cured ham.
[2] *n* indicates the number of replicates for each item within each treatment.

The concentration of standardized ileal digestible AAs (g/kg) in each product was calculated by multiplying the SID value (%) for each AA by the concentration (g/kg) of that AA in the product. This value was then divided by the concentration of CP in the product to calculate digestible indispensable AA content (mg) in 1 g protein (20). The digestible indispensable AA reference ratios were calculated for each product using Equation 4 (7).

$$\begin{aligned}&\text{Digestible indispensable AA reference ratio}\\&= \text{digestible indispensable AA content in 1 g protein of food (mg)}\\&\ /\text{mg of the same dietary indispensable AA in 1 g of reference protein.}\end{aligned} \quad (4)$$

Separate ratios were calculated using the reference protein for children from 6 mo to 3 y, and children older than 3 y, adolescents, and adults (7). The DIAAS were calculated using Equation 5 (7).

$$\text{DIAAS} = 100 \times \text{lowest value of digestible indispensable AA reference ratio.} \quad (5)$$

Statistical analysis

Normality of the data was tested by generating studentized residuals from each analysis. Outliers were removed until the Shapiro-Wilk test reached $P < 0.05$ and studentized residuals were within ±3 SD. Once outliers were removed, data were analyzed using PROC MIXED of SAS (SAS Institute Inc.) 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 AAs among products. 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 $0.05 \le P < 0.10$, respectively.

Results

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

AID

The AID of most AAs was not different between smoked and smoked-cooked bacon, with the exception that the AID of His and Trp was greater ($P < 0.05$) in smoked bacon than in smoked-cooked bacon and the AID of Arg, Ala, and Gly was greater ($P < 0.05$) in smoked-cooked bacon than in smoked bacon (**Table 3**). 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 His, Lys, Met, and Tyr was greater ($P < 0.05$) in raw belly than in smoked-cooked bacon. No differences in the AID of all AAs were observed between conventionally cured and alternatively cured ham, but conventionally cured and alternatively cured ham had greater AID ($P < 0.05$) of all AAs, except Tyr, than non-cured ham. The AID of all AAs, except Gly, was not different among the 3 processing temperatures for loin (i.e., 63°C, 68°C, and 72°C), and the AID of most AAs did not differ between the 2 bacon processing techniques and the 3 loin products. Raw belly had greater AID ($P < 0.05$) of Arg, His, Ile, Phe, Val, Asp, Cys, and Gly than 68°C and 72°C loins, whereas raw

TABLE 4 SID of AAs in the 9 pork products fed to growing pigs[1]

	Raw belly	Bacon		Ham			Loin			SEM	P value
		Smoked	Smoked-cooked	Non-cured	Alt. cured	Conv. cured	63°C	68°C	72°C		
n[2]	8	9	10	9	9	9	9	8	8		
Indispensable AAs											
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
Dispensable AAs											
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 AAs, %	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

[1] Values are means and pooled SEMs. Labeled means in a row without a common superscript letter differ, $P < 0.05$. AA, amino acid; alt. cured, alternatively cured ham; conv. cured, conventionally cured ham; SID, standardized ileal digestibility. SID 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.

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

belly, smoked-cooked bacon, and 63°C loin had greater AID ($P < 0.05$) of all AA, except Met and Tyr, than non-cured ham.

SID

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 4), but the SID of all AAs, except His, was not different between smoked and smoked-cooked bacon, and the SID of all AAs, 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 AAs, except Trp and Tyr, compared with non-cured ham. All other pork products had greater ($P < 0.05$) SID of all IAAs, except Arg and Lys, compared with non-cured ham. No difference in the SID of AAs was observed among the 3 processing temperatures for loin. Likewise, with the exception of His and Trp, no differences were observed in the SID of all IAAs among smoked bacon, smoked-cooked bacon, conventionally cured ham, alternatively cured ham, and the 3 loin products.

DIAAS

For children from 6 mo to 3 y (Table 5), loin heated to 63°C had the greatest ($P < 0.05$) DIAAS followed by smoked-cooked bacon. Alternatively cured ham had greater ($P < 0.05$) DIAAS than the other 2 ham products (conventionally cured and non-cured ham), but the conventionally cured and non-cured ham did not differ for DIAAS. Raw belly, smoked bacon, and 68°C and 72°C loins were not different for DIAAS and had reduced ($P < 0.05$) DIAAS 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 y, adolescents, and adults, smoked-cooked bacon had the greatest ($P < 0.05$) DIAAS followed by pork loin cooked to 63°C. 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 68°C and 72°C loins were not different for DIAAS and had the least ($P < 0.05$) DIAAS compared with the other pork products. The first limiting AA for all pork products for DIAAS was Val regardless of the processing method.

Discussion

The CP and AA composition in all pork products were generally within the range of published values (3). 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 cured with Prague powder the AA composition was similar. AA concentrations in the products used in this experiment were in agreement with published values (3).

Provisions of vitamins and micro minerals varied among diets, because different quantities of the pork products were included in the final diets. However, the pork products themselves also provided some vitamins and micro minerals (2) and because the inclusion of these nutrients in the N-free mixture was greater than the assumed requirement of the pigs,

TABLE 5 DIAAS for the 9 pork products as measured in growing pigs[1]

	Raw belly	Bacon		Ham			Loin			SEM	P value
		Smoked	Smoked-cooked	Non-cured	Alt. cured	Conv. cured	63°C	68°C	72°C		
n[2]	8	9	10	9	9	9	9	8	8		
Child 6 mo to 3 y[3]											
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	1.31	1.11	1.27	1.35	1.34	1.24	1.53	1.31	1.30		
AAA	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,[4] %	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
Older child, adolescent, and adult[5]											
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	1.54	1.30	1.50	1.58	1.57	1.46	1.80	1.54	1.53		
AAA	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,[4] %	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

[1]Values are means and pooled SEMs. Labeled means in a row without a common superscript letter differ, $P < 0.05$. AA, amino acid; AAA, aromatic amino acid; alt. cured, alternatively cured ham; conv. cured, conventionally cured ham; DIAA, digestible indispensable amino acid; DIAAS, digestible indispensable amino acid scores; SAA, sulfur amino acid.

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

[3]DIAAS were calculated using the recommended AA scoring pattern for a child (6 mo to 3 y). The indispensable AA reference patterns are expressed as mg AA/g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; SAA, 27; AAA, 52; Thr, 31; Trp, 8.5; Val, 40 (7).

[4]First-limiting AA is in parentheses.

[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; SAA, 23; AAA, 41; Thr, 25; Trp, 6.6; Val, 40 (7).

it is not likely that these vitamin and micro mineral differences influenced results. Likewise, the inclusion of titanium dioxide varied among diets because of the ways they were prepared, but the minimum inclusion level was 0.19% and there have been experiments published in which 0.25% titanium dioxide was used with no effect on digestibility of nutrients (21).

All diets were formulated to contain ∼16% CP to provide AAs that were close to the requirements of the pigs (16). Values for the SID of AAs are corrected for the basal endogenous losses of AAs and they are, therefore, independent of the CP in the diets because they are calculated based on the DM intake of each diet (19). As a consequence, values for DIAAS, which are based on SID values of AAs, are also independent of the CP in the diets. That SID values are independent of the amount of CP in the diet also has the consequence that these values are additive in mixed diets, whereas values for AID are not additive in mixed diets if 1 of the ingredients in the diet has less CP than the mixed diet (22). However, in the present experiment, each experimental diet contained only 1 source of protein, so the issue of additivity is not relevant here.

Before consumption, pork products almost always undergo processing (23), which is primarily carried out to develop sensorial qualities and inhibit the activity of pathogenic microorganisms (24–26). The effect of cooking on the structure of meat proteins has been widely studied, and the temperature and duration of cooking affects the extent of protein denaturation, oxidation, and aggregation (9, 27, 28). The cooking effect on protein and AA digestibility is not well described, but moderate protein denaturation occurs around 70–72°C, exposing protein cleavage sites to proteolytic enzymes resulting in increased digestibility (8, 9, 28). Hodgkinson et al. (10), observed increased protein quality when beef steaks were boiled to an internal temperature of 71°C. However, protein modification at temperatures of ≥100°C may result in protein oxidation and aggregation, which decrease digestibility (5, 28, 29).

In contrast with published data, the digestibility of AAs in the pork loins did not increase as the cooking temperature increased from 63°C to 72°C, and the digestibility of AAs in the 3 products was not different. However, the DIAAS decreased as temperature increased, which is likely the result of the lower concentration of digestible IAAs in 1 g of protein in the 68°C and 72°C loin compared with the loin heated to 63°C.

Curing, another form of processing, is characterized by the addition of salt, sodium or potassium nitrate or nitrite, sugar, and/or seasonings, which often results in the meat having a reddish-pink color (30). Nitrate or nitrite in combination with sodium chloride is largely used as a curing agent to inhibit the growth of *Clostridium botulinum* because of its antioxidant properties (26). 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 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 IAAs per gram of protein compared with conventionally cured and non-cured ham, resulting in greater DIAAS.

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 IAAs compared with the raw belly, which may be a result of overheating during cooking. The internal temperature of the bacon was not monitored during cooking, potentially resulting in overcooking. However, the USDA and Food Safety and Inspection Service recognize the difficulty in determining the internal temperature of bacon as a result of the thickness of the product and have suggested that if bacon is cooked crisp, a safe internal temperature has been reached (31). The decreased SID of IAAs in smoked-cooked bacon did not negatively affect the DIAAS because the greater DM content of this cooked product resulted in greater IAA concentrations per gram of protein, and this positively influenced DIAAS.

Regardless of processing method, all pork products evaluated in this experiment had DIAAS >100. Using DIAAS cutoffs, protein quality can be described as "Excellent," if DIAAS are >100 and "Good," if DIAAS are between 75 and 99 (7). Based on these cutoffs, all pork products used in this experiment can be described as "Excellent" quality proteins if consumed by children from 6 mo to 3 y or by individuals that are aged ≥3 y. Dairy proteins, fish proteins, and animal protein hydrolysates also have DIAAS >100, indicating that animal proteins, in general, are excellent quality proteins (32–34). Digestible indispensable amino acid scores >100 indicate that excellent quality protein has the potential to complement low-quality proteins (7). 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 (20). 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 >100, indicating that these proteins may complement low-quality proteins to produce a diet adequate in all IAAs. Results also indicated that various forms of processing did not negatively affect DIAAS, and on the contrary, curing and moderate heating of pork products may increase DIAAS.

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