RESEARCHREPORT
HUMAN NUTRITION

Title: **Determination of DIAAS and PDCAAS for pork burger, plant-based burgers, and the combined meal of burger bun and burgers – NPB #19-234**

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Industry Summary

Plant-based meat is available in human nutrition and has become more popular. Therefore, there has been an interest in the development of plant-based alternative proteins such as plant-based burgers. The DIAAS method is recommended as the best method to evaluate protein quality in human foods, although the PDCAAS method is also being used. However, there are no data for DIAAS or PDCAAS in plant-based burgers and it is not known how the protein quality in plant-based burgers compares with pork burger. Thus, the objective of this study was to test the hypothesis that pork protein has greater protein quality than plant protein included in plant-based burgers. The research was conducted by measuring amino acid digestibility in pigs and calculating DIAAS and PDCAAS values based on FAO recommendations. Seven diets were formulated using a pork burger, two plant burgers or a burger bun as the sole source of protein or combinations of pork or plant burgers and burger bun. Scores were calculated for toddlers from 6 months to 3 years old, preschool children from 2 to 5 years old, and for individuals older than 3 years. Results indicated that pork burger and the combination of pork burger and bun have greater protein quality than if plant-based burgers are consumed.

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Key Findings

- Pork burger and the combination of pork burger and bun have greater protein quality than plant-based burgers;
- There is no limiting AA in pork burger used as the sole source of protein for children older than 6 months;
- The combination of pork and burger bun provides a meal that is balanced in all indispensable AA.

Keywords: amino acid digestibility, DIAAS, PDCAAS, plant-based meat, pork.

Scientific Abstract

The demand for meat in developed and developing countries is increasing, but plant-based meat is also available. The digestible indispensable amino acid score (DIAAS) method has been recommended to evaluate protein quality in human foods, but the protein digestibility-corrected amino acid score (PDCAAS) method is used for regulatory purposes. There are, however, no values for DIAAS or PDCAAS in plant-based burgers and it is not known how the protein quality of plant-based burgers compares with pork burger. Thus, the objective of this study was to test the hypothesis that pork protein has greater quality than plant protein included in plant-based burgers. One pork burger (i.e., 80% lean), two plant-based burgers (i.e., Impossible Burger and Beyond Burger), and a burger bun were used. Seven diets were formulated. Four diets contained each food ingredient as the only source of crude protein (CP) and amino acids (AA). A N-free was also used and two diets were prepared by combining the pork burger or the Impossible Burger and burger bun. Diets were fed for 9 days to cannulated gilts and there were 6 replicates per diet. The initial 5 days were for adaptation, and fecal samples were collected in the mornings of days 6, 7, and 8. Ileal digesta were collected for 9h on days 8 and 9. The DIAAS values were calculated for children from 6 months to 3 years and for individuals older than 3 years, and PDCAAS values were calculated for preschool children from 2 to 5 years old. Results for DIAAS and PDCAAS indicated that for all age groups, the pork burger had greater ($P < 0.05$) scores than the plant-based burgers, and the combination of pork burger and bun also had greater ($P < 0.05$) scores than the combination of Impossible Burger and bun. In conclusion, results indicate that the pork burger and the combination of pork burger and bun have greater protein quality than plant-based burgers.

Introduction

In the U.S. and most other developed countries, animal-based proteins provide a significant portion of the human diet (Daniel et al., 2011). The demand for meat in developing countries continues to increase due to increasing animal protein consumption when income is available (Speedy, 2003). However, plant-based meat is also available and has become more popular due to its appearance, texture, and similar taste as meat products (Ruby, 2012). This trend is also driven by concerns involving animal welfare, human health, environmental impacts of animal production, religious practices, or personal preferences (Beardsworth and Kiel, 1991). Therefore, there has been an interest in the development of plant-based proteins. Examples of these proteins include two plant-based burgers, Impossible Burger and Beyond Burger.

To evaluate protein quality in human foods, the protein digestibility-corrected amino acid score **(PDCAAS)** method has been used (Food and Agriculture Organization: **FAO**, 1991), but some limitations with this procedure have been recognized (Mathai et al., 2017). Therefore, the digestible indispensable amino acid score **(DIAAS)** method is now recommended to better describe protein quality in human foods (FAO, 2013). The DIAAS procedure allows calculation of the protein value in mixed meals consisting of several proteins, indicating if individual food ingredients complement each other to produce a diet that is adequate in all indispensable amino acids **(AA)**, whereas for the PDCAAS procedure this is not possible. Moreover, the pig has been accepted as the preferred animal model for estimating AA digestibility (FAO, 2013).

Objective

The objective of this study was to test the hypothesis that pork protein has greater quality than plant protein included in plant-based burgers.

Materials and Methods

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

Animals, Housing, and Feeding

Ten gilts (initial body weight, **BW**: 24.6 ± 1.3 kg) were equipped with a T-cannula in the distal ileum (Stein et al., 1998). Following surgery, pigs were randomly allotted to a 10 × 6 Youden square design with 10 diets and six 9-d periods. All pigs were housed in individual pens (1.5 × 2.5 m) in an environmentally controlled room. Each pen had smooth sides and partially slatted floors. A nipple drinker and a feeder were also installed in each pen. All pigs were fed their assigned diets in a daily amount equivalent to 4% of BW for each pig. Daily feed allowances were provided in two equal meals at 0800 and 1700 h, and the amount of feed supplied each day was recorded. Water was available at all times. Pig weights were recorded at the beginning of each period to calculate feed allowance during the following period. At the conclusion of the experiment, pigs had a BW of 53.4 ± 6.6 kg.

Preparation of Ingredients and Diets

A pork burger and two plant-based burgers (i.e., Impossible Burger and Beyond Burger) were prepared at Colorado State University. All burgers were fully cooked to temperatures recommended by the United States Department of Agriculture **(USDA)** and the Food Safety and Inspection Service (**FSIS**; USDA-FSIS, 2012). A burger bun was procured from the Food Science & Human Nutrition Pilot Plant at University of Illinois **(Table 1)**. The four ingredients were included in one diet each as the sole source of crude protein **(CP)** and AA. Two additional diets of combined burgers (i.e., pork or Impossible Burger) and burger bun were prepared by combining one patty of burger (113g) and one burger bun (90g). A nitrogen-free **(N-free)** diet was used to measure basal endogenous losses of CP and AA to enable the calculation of standardized ileal digestibility **(SID)** of CP and AA and the calculation of DIAAS. The last 3 diets were unrelated to this work. Thus, seven diets were used in this study **(Tables 2 and 3)**. Titanium dioxide was included in the burger bun dough and in the N-free diet at 0.6% as an indigestible marker. The ingredients were fed with a premix that contained vitamins and minerals to meet or exceed current nutrient requirement estimates of swine (National Research Council; NRC, 2012).

Sample Collection

Experimental periods were 9 d with the initial 5 d for adaptation to the diet. Fecal samples were collected in the mornings on d 6, 7, and 8 and stored at - 20 °C. Ileal digesta were collected for 9 h on d 8 and 9 following standard procedures (Stein et al., 1998). In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed when filled with ileal digesta, or at least once every 30 minutes, and immediately frozen at – 20 °C to prevent bacterial degradation of AA in the digesta. On the completion of one experimental period, animals were deprived of feed overnight, and the following morning, the new experimental diet was offered.

Chemical Analysis

A sample of each food ingredient and of each diet was collected for chemical analysis. At the end of the experiment, fecal samples were dried and ground through a 1-mm screen in a Wiley Mill (model 4; Thomas Scientific, Swedesboro, NJ, USA) and ileal digesta were thawed, mixed within animal and diet, and a sub-sample was lyophilized and finely ground prior to chemical analysis. Samples of all ingredients, diets, fecal, and ileal digesta samples were analyzed for dry matter (**DM**, Method 927.05; AOAC International, 2007). Diets and ingredients were analyzed for ash (Method 942.05; 10) and N using the Kjeldahl method (Method 984.13; 17) on a KjeltecTM 8400 (FOSS Inc.). Nitrogen in ileal digesta and fecal samples was analyzed by combustion (Method 990.03; AOAC International, 2007) using a LECO FP628 analyzer (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as N × 6.25. Diets, ingredients, and ileal digesta samples were analyzed for AA [Method 982.30 E (a, b, c); AOAC International, 2007]. Ingredients were analyzed for gross energy using an isoperibol bomb calorimeter (Model 6300, Parr Instruments, Moline, IL, U.S.A.) with benzoic acid as the standard for calibration, and acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3N HCl (AnkomHCl, Ankom Technology, Macedon, NY, U.S.A.) followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY, U.S.A.). Calcium and phosphorus were also determined in the ingredients by inductively coupled plasma spectrometry method (Method 985.01 a, b, and c; AOAC Int., 2007) after wet ash sample preparation (Method 975.03 B[b]; AOAC Int., 2007). Titanium was analyzed for diets, fecal and ileal samples (Myers et al., 2004).

Calculations

Values for apparent ileal digestibility (**AID**), basal endogenous losses, and SID of CP and AA were calculated (Stein et al., 2007), and the standardized total tract digestibility (**STTD**) of CP was also calculated (Mathai et al., 2017). These values were used to calculate DIAAS and PDCAAS. The DIAAS reference ratio for each protein source was calculated using the following equation (FAO, 2013; Cervantes-Pahm et al., 2014):

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

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

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

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

Calculation of PDCAAS values used the reference protein for preschool children from 2 to 5 year old and were calculated using the following equation (FAO, 1991):

PDCAAS (%) = mg of limiting AA in 1 g of test protein = mg of the same AA in 1 g of reference protein × standardized total tract digestibility (%) × 100.

Statistical Analysis

At the conclusion of the experiment, normality of data was verified and outliers were identified using the UNIVERIATE and BOXPLOT procedures, respectively (SAS Institute Inc. Cary, NC, USA). Data were also analyzed by ANOVA using PROC MIXED procedure in SAS (SAS Institute Inc. Cary, NC, USA). The pig was the experimental unit for all analyses. Diet was the fixed effect, and pig and period were random effects. Treatment means were calculated using the LS MEANS statement in SAS, and if significant, means were separated using the PDIFF option in the MIXED procedure. An alpha value of 0.05 was used to assess significance among means.

Results and Discussion

All pigs remained healthy during the experimental period and only little food refusals were observed. The nutrient composition of pork was within the range of published values (USDA database) for cooked ground meat. Likewise, the nutrient composition of the plant-based burgers were within the range of published values (USDA database) for burger made from plants. The nutrient composition of the burger bun was also within the range of published values (USDA database) for branded burger buns.

AID/SID and ATTD/STTD

The AID for CP was greater ($P < 0.05$) in the pork burger compared with Beyond Burger, burger bun, and the combined meal of pork and bun **(Table 4)**. Likewise, the AID for Arg and Tyr was greater ($P < 0.05$) in all burgers and in the combined meal of Impossible Burger and bun than in the burger bun. The pork burger had greater ($P < 0.05$) AID for His, Ile, Leu, Thr, and Asp when compared to the other ingredients and meals. All burgers and combined meals had greater ($P < 0.05$) AID for Lys compared with the burger bun. In contrast, the burger bun had greater ($P < 0.05$) AID for Cys than the burgers and meals. The Beyond Burger had lower ($P < 0.05$) AID for Thr and Ala compared with the other ingredients and meals.

The pork burger had greater ($P < 0.05$) SID for His, Ile, Leu, Phe, Thr, Val, and Asp compared with the Beyond Burger, the burger bun, and the combined meals **(Table 5)**. Likewise, the SID of Met, Trp, and Tyr in pork burger was greater ($P < 0.05$) than the Beyond Burger, the burger bun, and the combination of Impossible Burger and bun. The burger bun had lower ($P < 0.05$) SID for Lys, but greater ($P < 0.05$) SID for Cys, compared with burgers and combined meals.

The ATTD and STTD of CP were lower ($P < 0.05$) in the plant-based burgers than in the pork burger and the burger bun, but the ATTD and STTD of CP in the combined meal of pork and bun were not different from the ATTD and STTD of CP in the combination of Impossible Burger and bun **(Table 6)**.

DIAAS

For both age groups, the pork burger had greater ($P < 0.05$) DIAAS compared with plant-based burgers **(Table 7)**. Likewise, the combination of pork burger and bun had greater ($P < 0.05$) DIAAS than the combined meal of Impossible Burger and bun. The Beyond Burger had lower ($P <$

0.05) DIAAS than the other burgers. The burger bun had the least ($P < 0.05$) DIAAS compared with all burgers and meals. There was no limiting AA (DIAAS > 100) in the pork burger for children from 6 mo to 3 yr, but in the plant burgers the first limiting AA was SAA, and in the combination of pork and bun the first limiting AA was Val. For individuals older than 3 yr, there was no limiting AA (DIAAS > 100) in the pork burger, in the combination of pork and bun, or in the Impossible Burger, but in the Beyond Burger, the first limiting AA was SAA. For both age groups, Lys was the first limiting AA in the burger bun and in the combination of Impossible Burger and bun. According to the FAO recommend AA patterns, the pork burger and the combination of pork and bun had DIAAS ≥ 100 qualifying as an "excellent" sources of protein for both age groups. The Impossible Burger also had DIAAS ≥ 100 for individuals older than 3 years.

The observation that DIAAS was > 100 in the pork burger for both age groups are in agreement with published values for DIAAS in pork products (Bailey et al., 2020). Likewise, the observation that DIAAS was < 100 in the plant burgers is in agreement with published values for DIAAS in soy protein isolate and pea protein concentrate (Mathai et al., 2017), and for cereal grains (Cervantes-Pahm et al., 2014). The fact that the Impossible Burger had DIAAS close to or greater than 100 for both age groups indicates that the ingredient composition of the Impossible Burger closer matches the required AA balance by humans than the combination of ingredients used to produce the Beyond Burger. In conclusion, data for DIAAS are in agreement with previous research and indicate that the pork burger has better protein quality than the plant-based burgers.

PDCAAS

For preschool children from 2 to 5 yr, the pork burger had greater ($P < 0.05$) PDCAAS compared with plant-based burgers **(Table 8)**. Likewise, the combination of pork burger and bun had greater ($P < 0.05$) PDCAAS than the combination of Impossible Burger and bun. The Beyond Burger had lower ($P < 0.05$) PDCAAS than the other burgers. The burger bun had the least ($P < 0.05$) PDCAAS compared with all burgers and meals. Values for PDCAAS were truncated to 100 for the pork burger as recommended by FAO (1991), and the first limiting AA was Trp. The first limiting AA in the Impossible Burger and Beyond Burger were Lys and Trp, respectively. The combined meal of pork burger and bun also had Trp as first limiting AA, whereas Lys was the first limiting AA in the burger bun and in the combination of Impossible Burger and bun. The results indicating that PDCAAS < 100 for preschool children in the plant based burgers are in agreement with published values for plant proteins (Schaafsma, 2000; Mathai et al., 2017; Abelilla et al., 2018). Therefore, the current data for PDCAAS are in agreement with results of previous research. As is the case when conclusions are based on DIAAS, the PDCAAS values also indicate that pork burger has better protein quality than plant-based burgers.

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Table 1. Analyzed nutrient composition of ingredients, as fed-basis

	Ingredients			
Item, %	Pork	Impossible	Beyond	Bun
DM	40.53	44.14	47.36	70.64
Gross energy (kcal/kg)	2,875	2,572	3,203	3,355
CP	19.62	18.10	20.22	12.10
AEE[1]	15.98	10.65	9.33	3.40
Ash	0.87	2.66	1.63	1.65
Minerals				
Ca	0.01	0.21	0.09	0.05
P	0.19	0.22	0.29	0.10
Indispensable AA				
Arg	1.29	1.11	1.63	0.42
His	0.62	0.42	0.50	0.24
Ile	0.90	0.87	1.00	0.45
Leu	1.48	1.35	1.69	0.78
Lys	1.55	1.02	1.36	0.28
Met	0.49	0.19	0.26	0.18
Phe	0.78	0.93	1.16	0.59
Thr	0.83	0.68	0.75	0.32
Trp	0.23	0.23	0.18	0.13
Val	0.97	0.94	1.12	0.50
Total	9.14	7.73	9.66	3.89
Dispensable AA				
Ala	1.16	0.75	0.88	0.36
Asp	1.71	1.91	2.23	0.52
Cys	0.19	0.35	0.27	0.25
Glu	2.57	3.58	3.18	3.69
Gly	1.29	0.73	0.80	0.41
Pro	0.93	0.82	0.89	1.21
Ser	0.70	0.72	0.96	0.50
Tyr	0.71	0.68	0.78	0.33
Total	9.27	9.54	9.98	7.27
Total amino acids	18.41	17.27	19.64	11.16

[1]AEE = acid hydrolyzed ether extract.

Table 2. Ingredient composition of experimental diets, as fed-basis[1]

	Diets						
Item, %	Pork	Impossible	Beyond	Bun	Pork + Bun	Impossible + Bun	N-free
Burger	55.10	55.10	52.50	-	37.10	37.10	-
Bun	-	-	-	90.60	29.60	29.60	-
Corn starch	12.83	12.83	15.52	-	6.25	6.25	67.67
Solka floc	4.00	4.00	4.00	-	4.00	4.00	4.00
Soy oil	4.00	4.00	4.00	0.30	4.00	4.00	4.00
Monocalcium phosphate	1.25	1.25	1.30	1.40	1.30	1.30	1.70
Limestone	1.17	1.17	1.03	1.00	1.10	1.10	0.98
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Magnesium oxide	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Potassium carbonate	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sucrose	20.00	20.00	20.00	5.05	15.00	15.00	20.00
Titanium dioxide	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15

[1]All diets, except N-free, were formulated to contain approximately 15% crude protein (DM basis).

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

Table 3. Analyzed nutrient composition of experimental diets, as fed-basis

	Diets						
Item, %	Pork	Impossible	Beyond	Bun	Pork + Bun	Impossible + Bun	N-free
Dry matter	65.50	67.09	69.91	71.02	67.35	70.05	91.96
Crude Protein	11.48	9.63	10.29	10.57	10.56	11.65	0.42
Ash	3.23	3.78	3.44	3.70	3.82	3.83	4.09
Indispensable AA							
Arg	0.67	0.59	0.76	0.33	0.59	0.55	0.01
His	0.36	0.23	0.24	0.21	0.31	0.24	0.00
Ile	0.51	0.46	0.48	0.38	0.46	0.46	0.01
Leu	0.83	0.72	0.80	0.67	0.79	0.76	0.02
Lys	0.91	0.57	0.69	0.22	0.69	0.49	0.01
Met	0.26	0.11	0.12	0.13	0.23	0.13	0.01
Phe	0.44	0.50	0.56	0.50	0.46	0.54	0.02
Thr	0.46	0.36	0.35	0.28	0.40	0.36	0.01
Trp	0.12	0.12	0.10	0.11	0.13	0.13	0.02
Val	0.54	0.49	0.53	0.42	0.50	0.50	0.01
Total	5.11	4.15	4.63	3.27	4.56	4.13	0.12
Dispensable A							
Ala	0.64	0.41	0.43	0.31	0.57	0.40	0.01
Asp	0.97	1.03	1.07	0.43	0.82	0.88	0.02
Cys	0.11	0.23	0.13	0.20	0.14	0.24	0.00
Glu	1.55	2.09	1.60	3.14	2.03	2.65	0.02
Gly	0.67	0.39	0.38	0.35	0.67	0.41	0.01
Pro	0.50	0.46	0.43	1.04	0.71	0.71	0.02
Ser	0.36	0.40	0.42	0.42	0.41	0.44	0.01
Tyr	0.24	0.28	0.31	0.19	0.33	0.29	0.01
Total	5.04	5.27	4.76	6.09	5.68	6.02	0.10
Total amino acids	10.15	9.42	9.39	9.36	10.24	10.15	0.22

Table 4. Apparent ileal digestibility (AID) of crude protein (CP) and amino acids (AA) in ingredients[1]

	Ingredients							
Item, %	Pork	Impossible	Beyond	Bun	Pork + Bun	Impossible + Bun	SEM	*P*-value
CP	89.6[a]	82.2[abc]	73.2[c]	79.0[bc]	78.1[bc]	83.6[ab]	3.25	0.028
Indispensable AA								
Arg	95.0[a]	91.6[a]	87.7[a]	74.0[b]	84.8[ab]	89.4[a]	3.72	0.015
His	93.7[a]	89.1[b]	83.5[c]	85.1[c]	88.8[b]	86.5[bc]	1.22	<0.001
Ile	92.1[a]	89.3[b]	85.4[c]	85.3[c]	87.8[bc]	86.8[bc]	1.03	0.0002
Leu	92.8[a]	89.2[b]	85.6[c]	87.8[bc]	89.5[b]	87.8[bc]	1.04	0.001
Lys	94.7[a]	89.5[ab]	88.8[ab]	46.7[c]	88.9[ab]	84.2[b]	3.03	<0.001
Met	94.9[a]	89.7[b]	79.3[c]	88.9[b]	92.9[a]	88.1[b]	0.96	<0.001
Phe	91.2	90.7	87.9	89.7	88.4	89.5	0.96	0.093
Thr	86.7[a]	79.4[bc]	74.1[cd]	73.4[d]	79.3[b]	75.2[bcd]	1.98	0.0001
Trp	96.6[a]	92.9[bc]	90.0[cd]	88.0[d]	94.2[ab]	92.0[bc]	1.41	0.001
Val	90.7[a]	87.4[ab]	83.0[c]	82.6[c]	85.6[bc]	84.7[bc]	1.26	0.001
Dispensable AA								
Ala	92.3[a]	84.7[ab]	76.5[bc]	68.8[c]	84.7[ab]	81.6[b]	3.16	0.001
Asp	90.9[a]	82.5[bc]	82.6[bc]	71.7[d]	84.1[b]	79.2[c]	1.73	<0.001
Cys	67.3[b]	70.7[b]	53.1[c]	84.5[a]	68.3[b]	71.3[b]	2.67	<0.001
Glu	93.6[ab]	92.8[ab]	87.3[c]	95.1[a]	92.3[b]	93.3[ab]	0.77	<0.001
Ser	86.4	84.0	79.2	83.5	81.4	83.1	1.78	0.117
Tyr	87.9[a]	88.8[a]	85.5[a]	79.9[b]	87.9[a]	86.4[a]	1.30	0.0004

[a-d] Mean values within a row with unlike superscript letters are different ($P < 0.05$).

[1]Data are least squares means of 6 observations per treatment, except for the Impossible and the bun that have 5 observations per treatment.

Table 5. Standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in ingredients[1,2]

	Ingredients							
Item, %	Pork	Impossible	Beyond	Bun	Pork + Bun	Impossible + Bun	SEM	*P*-value
CP	103.4	99.1	89.7	95.3	93.6	98.2	3.25	0.081
Indispensable AA								
Arg	105.5	103.9	97.7	97.1	97.1	103.3	3.72	0.392
His	97.7[a]	95.6[ab]	89.8[c]	92.4[bc]	93.6[b]	92.9[bc]	1.22	0.002
Ile	96.3[a]	94.1[ab]	90.1[c]	91.3[bc]	92.6[bc]	91.8[bc]	1.03	0.001
Leu	96.8[a]	93.9[b]	90.0[c]	93.1[b]	93.8[b]	92.4[bc]	1.04	0.001
Lys	98.6[a]	95.8[a]	94.2[a]	63.9[b]	94.1[a]	91.8[a]	3.03	<0.001
Met	97.0[a]	94.6[abc]	83.9[d]	93.0[bc]	95.2[ab]	92.5[c]	0.96	<0.001
Phe	95.9[a]	94.9[ab]	91.8[c]	94.1[abc]	93.0[bc]	93.6[abc]	0.96	0.042
Thr	95.1[a]	90.2[ab]	85.8[b]	88.3[b]	89.1[b]	86.7[b]	1.98	0.014
Trp	102.4[a]	99.0[ab]	97.8[bc]	94.8[c]	99.8[ab]	98.1[bc]	1.41	0.012
Val	96.1[a]	93.5[ab]	88.9[c]	90.2[bc]	91.7[bc]	91.0[bc]	1.26	0.003
Dispensable AA								
Ala	100.2	97.4	89.2	86.4	93.8	95.2	3.16	0.054
Asp	96.7[a]	88.1[bc]	88.1[bc]	85.7[c]	91.1[b]	86.0[c]	1.73	0.001
Cys	78.8[b]	76.5[b]	64.0[c]	91.5[a]	77.7[b]	77.0[b]	2.67	<0.001
Glu	98.0[a]	96.1[ab]	91.8[c]	97.4[ab]	95.7[b]	96.0[ab]	0.77	0.0001
Ser	96.9	93.9	88.9	93.3	90.9	92.3	1.78	0.058
Tyr	95.0[a]	95.2[ab]	91.5[bc]	89.7[c]	93.2[ab]	92.8[abc]	1.30	0.029

[a-c] Mean values within a row with unlike superscript letters are different (*P* < 0.05).

[1]Data are least squares means of 6 observations per treatment, except for the Impossible and the bun that have 5 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 of amino acids were calculated from pigs fed the N-free diet as follows (g/kg DM intake): Arg, 1.08; His, 0.22; Ile, 0.32; Leu, 0.50; Lys, 0.53; Met, 0.08; Phe, 0.31; Thr, 0.58; Trp, 0.11; Val, 0.45; Ala, 0.78; Asp, 0.85; Cys, 0.20; Glu, 1.02; Ser, 0.58; Tyr, 0.26.

Table 6. Apparent total tract digestibility (ATTD) and total tract digestibility (STTD) of crude protein (CP) in ingredients[1]

	Ingredients							
Item, %	Pork	Impossible	Beyond	Bun	Pork + Bun	Impossible + Bun	SEM	*P*-value
ATTD of CP	93.9[a]	88.6[c]	88.8[c]	93.0[ab]	92.3[ab]	90.3[bc]	1.06	0.002
STTD of CP	97.4[a]	92.9[c]	93.0[c]	97.1[a]	96.3[ab]	94.0[bc]	1.06	0.005

[a-c] Mean values within a row with unlike superscript letters are different ($P < 0.05$).

[1]Data are least squares means of 6 observations per treatment, except for the burger bun that has 5 observations per treatment.

Table 7. Digestible indispensable amino acid scores (DIAAS) in ingredients[1]

	Ingredient							
Item	Pork	Impossible	Beyond	Bun	Pork + Bun	Impossible + Bun	SEM	*P*-value
Child (6 mo to 3yr)[2]								
DIAA reference ratio								
His	1.55	1.12	1.11	0.92	1.31	1.03		
Ile	1.38	1.41	1.40	1.06	1.24	1.27		
Leu	1.11	1.06	1.14	0.91	1.02	0.99		
Lys	1.36	0.95	1.11	0.26	1.00	0.73		
SAA	1.17	0.91	0.71	1.21	1.14	0.97		
AAA	1.39	1.62	1.69	1.35	1.36	1.51		
Thr	1.29	1.09	1.02	0.75	1.06	0.94		
Trp	1.42	1.45	1.05	1.20	1.34	1.37		
Val	1.11	1.13	1.14	0.87	1.00	1.02		
DIAAS, %	111[a]	91[c] (SAA)	71[d] (SAA)	26[e] (Lys)	100[b] (Val)	73[d] (Lys)	2.01	<0.001
Older child, adolescent, adult[3]								
DIAA reference ratio								
His	1.94	1.40	1.39	1.15	1.63	1.29		
Ile	1.47	1.51	1.49	1.13	1.33	1.36		
Leu	1.20	1.14	1.23	0.98	1.10	1.07		
Lys	1.62	1.13	1.32	0.31	1.19	0.86		
SAA	1.38	1.07	0.83	1.42	1.34	1.14		
AAA	1.77	2.06	2.15	1.72	1.72	1.92		
Thr	1.60	1.35	1.27	0.93	1.32	1.16		
Trp	1.83	1.87	1.35	1.54	1.73	1.76		

Val	1.19	1.21	1.23	0.93	1.07	1.10		
DIAAS, %	119[a]	107[b]	83[c] (SAA)	31[d] (Lys)	107[b]	86[c] (Lys)	2.30	<0.001

[a-e] Mean values within a row with unlike superscript letters are different ($P < 0.05$).

[1]First-limiting AA is in parenthesis. AAA, aromatic AA; SAA, DIAA, digestible indispensable amino acid; SAA, sulfur AA.

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

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

Table 8. Protein digestibility corrected amino acid score (PDCAAS) in ingredients[1]

Item	Ingredient							
	Pork	Impossible	Beyond	Bun	Pork + Bun	Impossible + Bun	SEM	*P*-value
Children (2-5yr)								
IAA reference ratio								
His	1.63	1.14	1.22	1.01	1.41	1.10		
Ile	1.60	1.59	1.65	1.29	1.48	1.49		
Leu	1.11	1.05	1.18	0.95	1.05	1.01		
Lys	1.33	0.91	1.08	0.39	1.01	0.73		
SAA	1.34	1.11	0.97	1.38	1.34	1.19		
AAA	1.17	1.31	1.42	1.17	1.16	1.26		
Thr	1.21	1.02	1.01	0.76	1.05	0.93		
Trp	1.04	1.05	0.77	0.95	1.00	1.01		
Val	1.38	1.38	1.47	1.15	1.29	1.30		
PDCAAS[2], %	100[a] (Trp)	91[c] (Lys)	77[d] (Trp)	39[f] (Lys)	100[b] (Trp)	73[e] (Lys)	0.91	<0.001

[a-f] Mean values within a row with unlike superscript letters are different ($P < 0.05$).

[1]First-limiting AA is in parenthesis. AAA, aromatic AA; SAA, sulfur AA.

[2]PDCAAS were calculated using the recommended AA scoring pattern for children (2-5yr). The indispensable AA reference patterns are expressed as mg amino acid/g protein: His, 19; Ile, 28; Leu, 66; Lys, 58; SAA, 25; AAA, 63; Thr, 34; Trp, 11; Val, 35 (FAO, 1991).