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ORIGINAL CONTRIBUTION

Digestible indispensable amino acid score (DIAAS) is greater in animal-based burgers than in plant-based burgers if determined in pigs

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Abstract

Purpose Determine digestible indispensable amino acid score (DIAAS) for animal- and plant-based burgers and test the hypothesis that DIAAS calculated for a burger and a burger bun is additive in a combined meal.

Methods Ten ileal cannulated gilts were fed experimental diets for six 9-d periods with ileal digesta being collected on d 8 and 9 of each period. Six diets contained a burger (i.e., 80% lean beef, 93% lean beef, 80% lean pork, Impossible Burger, or Beyond Burger) or a burger bun as the sole source of crude protein and amino acids. Three additional diets were based on a combination of the bun and 80% beef, pork, or Impossible Burger. A nitrogen-free diet was also used. The DIAAS for all ingredients and mixed meals was calculated for children from 6 months to 3 years and for individuals older than 3 years, and DIAAS for combined meals was predicted from individual ingredient DIAAS.

Results The 93% lean beef and the pork burgers had greater ($P<0.05$) DIAAS than the plant-based burgers for both age groups. The 80% lean beef burger had greater ($P<0.05$) DIAAS than the plant burgers for children from 6 months to 3 years, and greater ($P<0.05$) DIAAS than the Beyond Burger for individuals older than 3 years. There were no differences between the measured and predicted DIAAS.

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

Keywords Amino acid digestibility · Animal-based meat · Digestible indispensable amino acid score · Plant-based meat · Protein quality

Abbreviations

AA	Amino acids
AAA	Aromatic amino acids
AEE	Acid hydrolyzed ether extract
AID	Apparent ileal digestibility
Asx	Sum of asparagine and aspartic acid
CP	Crude protein
DIAA	Digestible indispensable amino acid
DIAAS	Digestible indispensable amino acid score
Glx	Sum of glutamine and glutamic acid
SAA	Sulfur amino acids
SE	Standard error
SID	Standardized ileal digestibility

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Introduction

In the US and most other developed countries, animal-based proteins provide a significant portion of the human diet [1]. When income is available, the demand for meat in developing countries continues to increase due to increased animal protein consumption; however, the increased supply is limited to specific countries and areas of Asia and South America, and does not occur in African countries [2]. On average,

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European citizen have an annual consumption of 22 kg of animal-based proteins and 16 kg of plant-based proteins [3]. However, it is expected that animal protein consumption in Europe and North America will begin to decline by 2035 [4].

Plant-based proteins are available in the food supply chain and have become accepted as having an appearance, texture, and taste that is close to that of animal products [5]. The trend towards consuming more plant protein is also driven by concerns about animal welfare, human health, environmental impacts of animal production, religious practices, or personal preferences [6]. Therefore, there has been an interest in the development of plant-based proteins. Examples of these proteins include the Impossible Burger and the Beyond Burger, which are plant-based burgers made primarily from soy and pea protein, respectively.

The digestible indispensable amino acid score (DIAAS) has been recommended as the best method to determine protein quality in human foods. This method allows for calculation of the protein value of both individual ingredients and mixed meals consisting of several proteins [7]. Values for DIAAS obtained in milk and breakfast cereals are additive in mixed meals [8], and the principle of additivity is believed to be applicable to all types of combined meals, but additional research to demonstrate this is needed. Therefore, the objectives of this experiment were to determine DIAAS values for animal- and plant-based burgers and to test the hypothesis that DIAAS calculated for a burger and a burger bun is additive in a combined meal.

Materials and methods

Preparation of ingredients and diets

Three animal-based burgers (80% lean beef, 93% lean beef, and 80% lean pork), two plant-based burgers (Impossible Burger and Beyond Burger), and one burger bun were included in the experiment (Table 1). Burgers were made of a round and flat patty of ingredients presented in Appendix 1. The ground beef and the plant-based products were purchased from Sysco Wholesale Company, Denver, CO, whereas the ground pork was purchased from Walmart Retail Company, Fort Collins, CO. All products were purchased in bulk, then formed into patties using a patty forming machine (Formax F6, Mokena, IL, USA), and cooked in a smokehouse (Enviro-Pak: CVU-650E, Clackamas, OR) at 157 °C and 20% humidity to an internal temperature of 71 °C as recommended [9] at the Global Food Innovation Center at the Colorado State University, Fort Collins, CO. The burgers were allowed to cool for 30 min before being vacuum-packed and kept at − 20 °C until shipment to the University of Illinois, Urbana, IL, where the digestibility trial was conducted. The 80% and 93% lean meat burgers were chosen, because the 80% lean is most commonly consumed in restaurants and fast food establishments, whereas the 93% lean is a representative sample of leaner ground beef that many consumers select in grocery stores. The burger bun was produced at the Food Science and Human Nutrition Pilot Plant at the University of Illinois, Urbana, IL. Buns were baked at approximately 177 °C for 13–14 min using a turbo-flow gas convection oven (Imperial ICVG-2, Alton, IL, USA).

Each of the five burgers and the burger bun were included in one diet as the sole source of crude protein (CP) and amino acids (AA). Three additional diets were prepared by combining one patty (113 g) of burger (i.e., 80% lean beef, pork, or Impossible Burger) and a burger bun (90 g). A nitrogen-free diet was also prepared and used to determine basal endogenous losses of CP and AA to enable calculation of standardized ileal digestibility (SID) of CP and AA. Thus, a total of ten diets were used in this experiment (Tables 2, 3). Despite the recommended dietary protein content of 100 g/kg (dry matter basis) [10, 11], all diets were formulated to contain 150 g/kg CP (dry matter basis) to feed diets closer to the AA requirements of growing pigs. For all diets, premix mixtures containing corn starch, solka floc, soy oil, sucrose, titanium dioxide, vitamins, and minerals were prepared to meet or exceed current nutrient requirement estimates for swine [12]. With the exception of the nitrogen-free diet, the premix mixtures for all diets were mixed separately to allow for combination with ingredients before feeding. The titanium dioxide as indigestible marker was calculated in the premix mixtures to result in 0.60% titanium dioxide in the final diets. The burger bun dough also contained 0.60% titanium dioxide.

Ethical considerations

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

Animals, housing, and feeding

Ten gilts (offspring of line 359 males mated to Camborough females; PIC, Henderson, TN, US) with an initial average body weight of 24.6 ± 1.3 kg were equipped with a T-cannula in the distal ileum [10, 13]. Following surgery, pigs were allowed 7 days to recuperate and a grower diet based on corn and soybean meal was fed during this time. Pigs were then randomly allotted to a 10×6 Youden square design with ten diets and six 9-day periods. No pig received the same diet more than once during the experiment and there were, therefore, six replicate pigs per treatment, which is the

Table 1 Analyzed nutrient composition of food ingredients (as fed-basis)

Item, %	80% lean beef	93% lean beef	Pork	Impossible burger	Beyond burger	Burger bun
Dry matter	44.52	41.34	40.53	44.14	47.36	70.64
Gross energy (kcal/kg)	3220	2720	2875	2572	3203	3355
Crude protein	24.04	27.61	19.62	18.10	20.22	12.10
AEE	16.92	11.59	15.98	10.65	9.33	3.40
Ash	0.70	1.55	0.87	2.66	1.63	1.65
Minerals						
Calcium	0.01	0.01	0.01	0.21	0.09	0.05
Phosphorus	0.15	0.18	0.19	0.22	0.29	0.10
Indispensable AA						
Histidine	0.65	0.85	0.62	0.42	0.50	0.24
Isoleucine	1.02	1.34	0.90	0.87	1.00	0.45
Leucine	1.73	2.20	1.48	1.35	1.69	0.78
Lysine	1.79	2.32	1.55	1.02	1.36	0.28
Methionine	0.54	0.72	0.49	0.19	0.26	0.18
Phenylalanine	0.93	1.14	0.78	0.93	1.16	0.59
Threonine	0.92	1.19	0.83	0.68	0.75	0.32
Tryptophan	0.25	0.33	0.23	0.23	0.18	0.13
Valine	1.15	1.38	0.97	0.94	1.12	0.50
Total	8.98	11.47	7.85	6.63	8.02	3.47
Dispensable AA						
Alanine	1.46	1.56	1.16	0.75	0.88	0.36
Arginine	1.50	1.74	1.29	1.11	1.63	0.42
Asx	1.96	2.48	1.71	1.91	2.23	0.52
Cystein	0.23	0.29	0.19	0.35	0.27	0.25
Glx	3.09	3.94	2.57	3.58	3.18	3.69
Glycine	1.74	1.35	1.29	0.73	0.80	0.41
Proline	1.22	1.10	0.93	0.82	0.89	1.21
Serine	0.80	0.96	0.70	0.72	0.96	0.50
Tyrosine	0.80	1.01	0.71	0.68	0.78	0.33
Total	12.80	14.43	10.55	10.65	11.62	7.69
Total AA	21.78	25.90	18.41	17.28	19.64	11.16

AA amino acids, *AEE* acid hydrolyzed ether extract, *Asx* sum of asparagine and aspartic acid, *Glx* sum of glutamine and glutamic acid

recommended number of pigs to have sufficient power of DIAAS experiments [10, 11]. All pigs were housed in individual pens (1.5 × 2.5 m) in an environmentally controlled room. Each pen had smooth sides and partially slatted floors. A nipple drinker and a feeder were also installed in each pen.

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

Sample collection

Although it is recognized that 7-day experimental periods are most often used [11], for practical reasons, experimental periods in this experiment were 9 days with the initial 7 days being the adaptation to each diet. Ileal digesta were collected for 9 h on days 8 and 9 following standard procedures with slight

Table 2 Ingredient composition of the 10 experimental diets (as fed-basis)

Item, %	80% lean beef	93% lean beef	Pork	Impossible burger	Beyond burger	Burger bun	80% lean beef + bun	Pork + bun	Impossible burger + bun	Nitrogen-free
Burger	42.80	42.80	55.10	55.10	52.50	–	32.50	37.10	37.10	–
Burger bun	–	–	–	–	–	90.60	25.90	29.60	29.60	–
Corn starch	25.05	25.05	12.83	12.83	15.52	–	9.49	6.25	6.25	67.67
Solka floc	4.00	4.00	4.00	4.00	4.00	–	4.00	4.00	4.00	4.00
Soy oil	4.00	4.00	4.00	4.00	4.00	0.30	4.00	4.00	4.00	4.00
Monocalcium phosphate	1.40	1.40	1.25	1.25	1.30	1.40	1.40	1.30	1.30	1.70
Limestone	1.10	1.10	1.17	1.17	1.03	1.00	1.06	1.10	1.10	0.98
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Magnesium oxide	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Potassium carbonate	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sucrose	20.00	20.00	20.00	20.00	20.00	5.05	20.00	15.00	15.00	20.00
Titanium dioxide	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Vitamin mineral premix[a]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

All diets, except the nitrogen-free, were formulated to contain approximately 15% crude protein (dry matter basis)

[a]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 the 10 experimental diets (as fed-basis)

Item, %	80% lean beef	93% lean beef	Pork	Impossible burger	Beyond burger	Burger bun	80% lean beef + bun	Pork + bun	Impossible burger + bun	Nitrogen-free
Dry matter	74.12	73.06	65.50	67.09	69.91	71.02	73.09	67.35	70.05	91.96
Crude protein	9.68	12.08	10.19	9.63	10.29	10.57	8.52	10.56	10.36	0.42
Ash	3.10	3.28	3.23	3.78	3.44	3.70	3.92	3.82	3.83	4.09
Indispensable AA										
Histidine	0.29	0.37	0.36	0.23	0.24	0.21	0.23	0.31	0.24	0.00
Isoleucine	0.48	0.57	0.51	0.46	0.48	0.38	0.38	0.46	0.46	0.01
Leucine	0.81	0.94	0.83	0.72	0.80	0.67	0.66	0.79	0.76	0.02
Lysine	0.87	1.04	0.91	0.57	0.69	0.22	0.58	0.69	0.49	0.01
Methionine	0.23	0.29	0.26	0.11	0.12	0.13	0.19	0.23	0.13	0.01
Phenylalanine	0.43	0.49	0.44	0.50	0.56	0.50	0.39	0.46	0.54	0.02
Threonine	0.43	0.50	0.46	0.36	0.35	0.28	0.33	0.40	0.36	0.01
Tryptophan	0.09	0.15	0.12	0.12	0.10	0.11	0.10	0.13	0.13	0.02
Valine	0.50	0.59	0.54	0.49	0.53	0.42	0.40	0.50	0.50	0.01
Total	4.13	4.94	4.43	3.56	3.87	2.92	3.26	3.97	3.61	0.11
Dispensable AA										
Alanine	0.59	0.69	0.64	0.41	0.43	0.31	0.47	0.57	0.40	0.01
Arginine	0.62	0.72	0.67	0.59	0.76	0.33	0.48	0.59	0.55	0.01
Asx	0.92	1.07	0.97	1.03	1.07	0.43	0.67	0.82	0.88	0.02
Cystein	0.10	0.12	0.11	0.23	0.13	0.20	0.11	0.14	0.24	0.00
Glx	1.50	1.78	1.55	2.09	1.60	3.14	1.73	2.03	2.65	0.02
Glycine	0.54	0.59	0.67	0.39	0.38	0.35	0.53	0.67	0.41	0.01
Proline	0.44	0.49	0.50	0.46	0.43	1.04	0.60	0.71	0.71	0.02
Serine	0.34	0.39	0.36	0.40	0.42	0.42	0.33	0.41	0.44	0.01
Tyrosine	0.31	0.35	0.24	0.28	0.31	0.19	0.26	0.33	0.29	0.01
Total	5.36	6.20	5.71	5.88	5.53	6.41	5.18	6.27	6.57	0.11
Total AA	9.49	11.14	10.14	9.44	9.40	9.33	8.44	10.24	10.18	0.22

AA amino acids, *Asx* sum of asparagine and aspartic acid, Glx sum of glutamine and glutamic acid modifications [10, 13]. 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 min, and immediately frozen at – 20 °C to prevent bacterial degradation of AA in the digesta. Cleaning of cannulas was completed every 24 h [10]. On the completion of one experimental period, animals were deprived of feed overnight, and in the following morning, a new experimental diet was offered. All pigs remained healthy during the experimental period and had a final average body weight of 53.4 ± 6.6 kg.

Chemical analysis

A sample of each food ingredient and of each diet was collected for chemical analysis. At the end of the experiment, ileal digesta were thawed, homogenized for each animal and diet, and a sub-sample was lyophilized and ground through a 0.5 mm screen prior to chemical analysis. Sub-samples of ingredients and diets were also lyophilized and ground through a 0.5 mm screen before analysis. All ingredients, diets, and ileal digesta samples were analyzed for dry matter (Method 927.05) [14] and for N by combustion (Method 990.0) [14] using a LECO analyzer (Model FP628, LECO Corp., Saint Joseph, MI, USA). Amino acids were analyzed on a Hitachi Amino Acid Analyzer (Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA, USA) after hydrolysis with 6 *N* HCl for 24 h at 110 °C (Method 982.30 E a, b, and c) [14]. Crude protein was calculated as N × 6.25. Diets and ingredient samples were also analyzed for ash (Method 942.05; 10) [14]. Ingredients were analyzed for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments Co., Moline, IL, USA) with benzoic acid as the standard for calibration, and acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3 *N* HCl (Model Ankom^{HCl}, Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (Model Ankom^{XT15}, Ankom Technology, Macedon, NY, USA). Calcium and phosphorus were also determined in all ingredients (Method 985.01 a, b, and c) [14] using inductively coupled plasma-optical emission spectrometry (ICP-OES; Model Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; 10) [14] and wet digestion with nitric acids (method 3050 B) [15]. Titanium was analyzed for all diets and ileal digesta samples following the procedure of Myers et al. [16].

Calculations

The apparent ileal digestibility (AID) of AA in each diet was calculated using the following equation [17]:

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

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

The basal endogenous flow to the distal ileum of each AA was determined based on the flow obtained after feeding the nitrogen-free diet using the following equation [17]:

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

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

By correcting the AID for the basal endogenous loss of CP or AA, SID was calculated using the following equation [17]:

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

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

The predicted AID of AA in the combined diet containing each burger and burger bun was calculated using the following equation [18]:

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

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

The digestible indispensable amino acid (DIAA) reference ratios as well as DIAAS values for each ingredient or mixed diet were calculated as previously explained [8] and as recommended by the Food and Agricultural Organization [7]. Separate reference ratios were calculated for children from 6 months to 3 years, and for older children, adolescents, and adults.

Statistical analysis

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

Results

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

The AID of CP, and some AA was greater ($P < 0.05$) in the 93% lean beef burger and in the pork burger than in the 80% lean beef burger and the Beyond Burger or the Impossible Burger (Table 4). All burgers had greater ($P < 0.05$) AID for lysine compared with the burger bun. The SID of CP, histidine, isoleucine, leucine, methionine, and valine was greater ($P < 0.05$) in the 93% lean beef, the pork, and the Impossible Burger than in the Beyond Burger, but the burger bun had lower ($P < 0.05$) SID of lysine compared with all burgers. The SID of valine and the sum of asparagine and aspartic acid (Asx) was greater ($P < 0.05$) in the pork burger than in the 80% lean beef burger, the Beyond Burger, and the burger bun.

Differences between the measured and the predicted AID and SID in the combined meal of 80% lean beef burger and bun differed ($P < 0.05$) from zero only for tryptophan (Table 5). For the combination of pork burger and bun, the AID and SID differed ($P < 0.05$) from zero for cysteine (Table 6). For the combined meal of Impossible Burger and bun, no differences between the measured and the predicted values for AID or SID were observed (Table 7).

For children from 6 months to 3 years and for individuals older than 3 years, the 93% lean beef and pork burgers had greater ($P < 0.05$) DIAAS compared with the other burgers, whereas the Beyond Burger had the lowest ($P < 0.05$) DIAAS for all burgers (Table 8). The animal-based burgers had greater ($P < 0.05$) DIAAS than the plant-based burgers for children between 6 months and 3 years. However, for children older than 3 years, adolescents, and adults, no differences were observed between the 80% lean beef and Impossible Burger, but the 80% lean beef had greater ($P < 0.05$) DIAAS than the Beyond Burger for this age group. The burger bun had lower ($P < 0.05$) DIAAS compared with all burgers. There was no limiting AA (DIAAS > 100) for the animal-based burgers for children from 6 months to 3 years, but for both plant-based burgers, the first limiting AA was the sulfur amino acids (SAA). For older children, adolescents and adults, there was no limiting AA (DIAAS > 100) for the animal-based burgers or for the Impossible Burger, but for the Beyond Burger, the first limiting AA was SAA. For both age groups, lysine was the first limiting AA in the burger bun.

For children from 6 months to 3 years, valine was the first limiting AA for the measured values of the combined meal of 80% lean beef burger and bun, but lysine was the first limiting AA for the predicted values, and there was no limiting AA for the combination of pork burger and bun (Table 9). For individuals older than 3 years, there was no limiting AA for the animal burger and bun combinations. However, for the combination of Impossible Burger and bun, lysine was the first limiting AA for both age groups. Differences between the measured and the predicted DIAAS values for the combination of 80% lean beef burger and bun differed ($P < 0.05$) from zero for tryptophan for both age groups. Likewise, differences between the measured and the predicted DIAAS values for the combination of pork burger and bun differed ($P < 0.05$) from zero for SAA. For the Impossible Burger and bun, no differences between the measured and the predicted DIAAS values were observed. Regardless of age group and burger–bun combination, there were no differences between the measured and the predicted DIAAS.

Discussion

In recent years, DIAAS for cereal grains [19–21], breakfast cereals [8, 22], pulse crops [22, 23], soy protein [22, 23], pistachio nuts [24], milk proteins [8, 22, 23], and meats [25–27] have been published. All these values are important to build a nutrient database required to formulate diets

Table 4 Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of CP and AA in food ingredients

Item, %	AID								SID							
	80% lean beef	93% lean beef	Pork	Impossible burger	Beyond burger	Burger bun	SE	P value	80% lean beef	93% lean beef	Pork	Impossible burger	Beyond burger	Burger bun	SE	P value
Crude protein	73.3^{c}	85.8^{ab}	88.2^{a}	82.2^{abc}	73.2^{c}	79.0^{bc}	3.14	0.006	91.9^{bc}	100.5^{ab}	103.8^{a}	99.1^{ab}	89.7^{c}	95.2^{abc}	3.14	0.024
Indispensable AA																
Histidine	87.5^{cd}	91.6^{ab}	93.7^{a}	89.1^{bc}	83.5^{e}	85.1^{de}	1.27	<0.001	93.1^{bc}	96.0^{ab}	97.7^{a}	95.6^{ab}	89.8^{c}	92.4^{bc}	1.27	0.002
Isoleucine	88.8^{b}	91.5^{ab}	92.1^{a}	89.3^{ab}	85.4^{c}	85.3^{c}	1.05	0.0002	93.8^{ab}	95.7^{a}	96.3^{a}	94.1^{ab}	90.1^{c}	91.3^{bc}	1.05	0.001
Leucine	89.6^{bc}	92.4^{ab}	92.8^{a}	89.2^{c}	85.6^{d}	87.8^{cd}	1.03	0.0002	94.2^{ab}	96.2^{a}	96.8^{a}	93.9^{ab}	90.0^{c}	93.2^{b}	1.03	0.001
Lysine	91.8^{a}	94.0^{a}	94.7^{a}	89.5^{a}	88.8^{a}	46.7^{b}	2.99	<0.001	96.3^{a}	97.7^{a}	98.6^{a}	95.8^{a}	94.2^{a}	63.9^{b}	2.99	<0.001
Methionine	93.3^{a}	95.0^{a}	94.9^{a}	89.8^{b}	79.3^{c}	88.9^{b}	0.85	<0.001	95.8^{a}	97.0^{a}	97.0^{a}	94.7^{ab}	83.9^{c}	93.0^{b}	0.85	<0.001
Phenylalanine	87.4	90.5	91.2	90.7	87.9	89.7	1.14	0.100	92.7	95.1	95.9	94.9	91.8	94.1	1.14	0.109
Threonine	78.9^{c}	85.0^{ab}	86.7^{a}	79.4^{bc}	74.1^{c}	73.4^{c}	2.08	0.0003	88.9^{bc}	93.4^{ab}	95.1^{a}	90.2^{abc}	85.8^{c}	88.3^{bc}	2.08	0.031
Tryptophan	85.9^{d}	94.7^{ab}	96.6^{a}	92.8^{abc}	90.0^{bcd}	87.9^{cd}	1.92	0.001	94.9^{b}	100.1^{a}	102.4^{a}	99.0^{ab}	97.8^{ab}	94.8^{b}	1.92	0.023
Valine	85.5^{bc}	89.6^{a}	90.7^{a}	87.4^{ab}	83.0^{c}	82.6^{c}	1.40	0.001	92.1^{bcd}	95.2^{ab}	96.1^{a}	93.5^{abc}	88.9^{d}	90.2^{cd}	1.40	0.006
Mean	88.1^{c}	91.5^{ab}	92.4^{a}	88.4^{bc}	84.9^{d}	82.5^{d}	1.18	<0.001	93.7^{bc}	96.1^{ab}	97.0^{a}	94.3^{ab}	90.5^{cd}	90.0^{d}	1.18	0.001
Dispensable AA																
Alanine	83.9^{ab}	90.9^{a}	92.3^{a}	84.7^{ab}	76.5^{bc}	68.8^{c}	3.08	0.0002	93.7^{abc}	99.2^{a}	100.2^{a}	97.4^{ab}	89.2^{bc}	86.4^{c}	3.08	0.023
Arginine	84.5^{b}	92.7^{ab}	95.0^{a}	91.6^{ab}	87.7^{ab}	74.0^{c}	3.56	0.006	97.4	103.8	105.5	103.9	97.7	97.1	3.56	0.322
Asx	84.2^{bc}	89.3^{ab}	90.9^{a}	82.5^{c}	82.6^{c}	71.7^{d}	1.80	<0.001	91.1^{bc}	95.1^{ab}	96.7^{a}	88.1^{cd}	88.1^{cd}	85.7^{d}	1.80	0.001
Cystein	48.4^{c}	62.2^{bc}	67.3^{b}	70.8^{ab}	53.1^{c}	84.6^{a}	4.94	0.0004	62.9^{c}	73.7^{bc}	78.8^{ab}	76.6^{bc}	64.0^{c}	91.6^{a}	4.94	0.004
Glx	88.9^{b}	92.8^{a}	93.6^{a}	92.8^{a}	87.3^{b}	95.1^{a}	1.08	0.0001	93.9^{bc}	97.0^{ab}	98.0^{a}	96.1^{ab}	91.8^{c}	97.4^{a}	1.08	0.002
Glycine	53.1^{c}	78.4^{ab}	87.9^{a}	70.9^{ab}	49.2^{c}	54.6^{c}	8.23	0.012	83.8	105.9	109.7	109.3	89.7	100.2	8.23	0.149
Serine	74.0^{b}	84.0^{a}	86.4^{a}	84.1^{a}	79.2^{ab}	83.5^{a}	2.69	0.030	86.6	94.7	96.9	93.9	88.9	93.3	2.69	0.084
Tyrosine	86.0^{ab}	89.8^{a}	87.9^{ab}	88.8^{ab}	85.5^{b}	79.9^{c}	1.44	0.001	92.3^{ab}	95.3^{a}	95.0^{a}	95.2^{a}	91.5^{ab}	89.7^{b}	1.44	0.039
Mean	80.9^{b}	88.9^{a}	91.1^{a}	86.7^{ab}	81.0^{b}	85.9^{ab}	2.09	0.007	91.5^{b}	97.9^{a}	99.9^{a}	95.4^{ab}	90.6^{b}	95.2^{ab}	2.09	0.025
Total AA	84.2^{b}	90.1^{a}	91.7^{a}	87.4^{ab}	82.6^{b}	84.7^{b}	1.65	0.003	92.5^{bc}	97.1^{ab}	98.5^{a}	95.0^{abc}	90.5^{c}	93.3^{bc}	1.65	0.017

The SID values were calculated by correcting values for basal ileal endogenous losses (g/kg dry matter intake): Crude protein 24.25, Arginine 1.08, Histidine 0.22, Isoleucine 0.32, Leucine 0.50, Lysine 0.53, Methionine 0.08, Phenylalanine 0.31, Threonine 0.58, Tryptophan 0.11, Valine 0.45, Alanine 0.78, Asx 0.85, Cystein 0.20, Glx 1.02, Serine 0.58, and Tyrosine 0.26

AA amino acids, *Asx* sum of asparagine and aspartic acid, *Glx* sum of glutamine and glutamic acid, *SE* standard error

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

Table 5 Measured and predicted values for apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein and amino acids (AA) in the 80% lean beef burger and bun meal

	AID				SID			
Item, %	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE
Crude protein	74.9	74.9	0.0	4.57	95.7	92.8	2.9	4.57
Indispensable AA								
Histidine	86.8	86.9	− 0.1	1.69	93.8	92.9	0.9	1.69
Isoleucine	86.7	87.9	− 1.2	1.28	93.0	93.2	− 0.2	1.28
Leucine	88.7	89.2	− 0.5	1.13	94.3	93.9	0.3	1.13
Lysine	87.5	86.9	0.6	1.53	94.2	92.8	1.4	1.53
Methionine	92.3	92.4	− 0.1	0.70	95.3	95.2	0.0	0.70
Phenylalanine	87.5	88.1	− 0.7	1.26	93.3	93.2	0.1	1.26
Threonine	76.9	77.7	− 0.9	2.67	89.8	88.8	1.0	2.67
Tryptophan	94.5	86.5	8.0**	1.42	102.6	94.9	7.7**	1.42
Valine	83.7	84.8	− 1.1	1.67	91.8	91.6	0.2	1.67
Mean	86.6	86.8	− 0.3	1.41	93.5	92.8	0.7	1.41
Dispensable AA								
Alanine	83.7	81.5	2.2	2.39	95.8	92.5	3.3	2.39
Arginine	85.2	82.6	2.6	3.35	101.7	97.4	4.4	3.35
Asx	82.3	82.1	0.3	2.26	91.6	90.2	1.5	2.26
Cystein	63.8	64.8	− 1.1	3.59	76.8	75.9	0.8	3.59
Glx	92.0	91.9	0.2	1.05	96.4	95.6	0.8	1.05
Glycine	67.5	53.4	14.2	7.07	98.3	86.3	11.9	7.07
Serine	78.9	77.1	1.7	3.04	91.7	88.8	2.9	3.04
Tyrosine	86.4	84.5	1.8	1.42	93.7	91.7	2.1	1.42
Mean	84.3	82.6	1.7	2.49	95.4	92.7	2.7	2.49
Total AA	85.2	84.3	0.9	2.03	94.6	92.7	1.9	2.03

Asx sum of asparagine and aspartic acid, *Glx* sum of glutamine and glutamic acid, *SE* standard error

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

for humans based on digestibility of AA [8]. However, because proteins are usually consumed in meals consisting of several food items, it is important to demonstrate that values for DIAAS are additive in combined meals, which allow for calculation of DIAAS in combinations of food ingredients with individually determined values for DIAAS. In addition to providing values for DIAAS in animal-based and plant-based burgers, results of the present experiment also provide data to demonstrate the additivity of DIAAS values, thus demonstrating the applicability of the DIAAS concept in practical nutrition.

Nutrient compositions of the animal-based burgers were within the range of published values [28] for cooked ground meat. Likewise, nutrient compositions of both plant-based burgers were within the range of published values for burgers made from plants [29]. The nutrient composition of the burger bun was also within the range of published values for branded burger buns [29]. The AID and SID of CP and AA that were determined for the beef burgers and the pork burger were within the range of reported values for pork loin and grilled topside steak, respectively [25, 26]. The SID for the Impossible Burger and the Beyond Burger were in agreement with SID values for soy protein isolate and pea protein concentrate, respectively [23].

Nutritional losses of essential AA as well as the formation of Maillard reaction products are undesirable effects of protein–carbohydrate complexes when processing foods in the presence of heat [30]. The Maillard reaction results in reduced concentration and digestibility of lysine and sometimes other AA [31, 32]. Because there is a high concentration of threonine in mucin, which is the major component of endogenous protein lost at the end of the small intestine [33], the AID of threonine is expected to be the lowest among all indispensable AA, which was also the case for all burgers. However, the burger bun had a lower AID of lysine than of threonine indicating that baking at 177 °C for 13–14 min may have caused heat damage to the bun, because temperature and time of processing have a significant influence on destruction of lysine [30, 32]. It is not uncommon that cooking or baking results in heat damage and, therefore, low SID

Table 6 Measured and predicted values for apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein and amino acids (AA) in the pork burger and bun meal

	AID				SID			
Item, %	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE
Crude protein	78.1	86.1	− 7.9	4.76	93.6	100.7	− 7.1	4.76
Indispensable AA								
Histidine	88.8	91.7	− 2.9+	1.38	93.6	96.5	− 2.9+	1.38
Isoleucine	87.8	90.2	− 2.4+	1.10	92.6	94.9	− 2.3+	1.10
Leucine	89.5	91.4	− 1.9	0.97	93.8	95.7	− 2.0	0.97
Lysine	88.9	88.7	0.3	1.54	94.1	94.2	0.0	1.54
Methionine	92.9	93.6	− 0.7	0.68	95.2	96.1	− 0.9	0.68
Phenylalanine	88.4	90.6	− 2.2+	1.06	93.0	95.2	− 2.2+	1.06
Threonine	79.3	83.6	− 4.3+	1.66	89.1	93.5	− 4.3+	1.66
Tryptophan	94.2	93.9	0.3	1.55	99.8	100.0	− 0.2	1.55
Valine	85.6	88.4	− 2.7+	1.31	91.7	94.4	− 2.7+	1.31
Mean	87.9	89.9	− 2.0	1.21	93.1	95.2	− 2.1	1.21
Dispensable AA								
Alanine	84.7	87.7	− 3.0	3.12	93.8	97.5	− 3.7	3.12
Arginine	84.8	90.7	− 5.9	4.66	97.1	103.8	− 6.6	4.66
Asx	84.1	87.2	− 3.2	1.91	91.1	94.6	− 3.5	1.91
Cystein	68.3	76.2	− 7.9*	2.06	77.7	85.3	− 7.7*	2.06
Glx	92.3	94.4	− 2.1	1.10	95.7	97.7	− 2.0	1.10
Glycine	72.4	81.1	− 8.7	8.91	94.8	107.8	− 13.0	8.91
Serine	81.4	85.3	− 4.0	2.31	90.9	95.6	− 4.7+	2.31
Tyrosine	87.9	85.8	2.1	1.18	93.2	93.6	− 0.4	1.18
Mean	85.4	89.1	− 3.7	2.81	93.9	98.1	− 4.1	2.81
Total AA	86.5	89.4	− 3.0	2.11	93.6	96.8	− 3.3	2.11

Asx sum of asparagine and aspartic acid, *Glx* sum of glutamine and glutamic acid, *SE* standard error

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

of lysine [8, 22]. As a consequence, the SID of lysine is often low in meals prepared from different baked foods [34, 35].

The few differences observed between the measured and the predicted SID values of AA for the combination of animal-based burgers and bun, and none for the combination of Impossible Burger and bun, demonstrated that values based on SID generally were additive in the combined meals. This is in agreement with data reported for other mixed diets [8, 18, 36]. The reason measured values for AID of CP were not different from predicted values is that concentrations of CP in diets based on individual ingredients were not different from the CP of the combined meals. As a consequence, there was no underestimation of AID in individual ingredients and AID values were, therefore, as additive as values for SID [18]. However, if at least one low-protein food is used in a mixed meal, it is more accurate to use SID values to predict the digestibility for CP and AA in mixed meals [18, 36]. The difference between measured and calculated AID and SID for tryptophan in the 80% lean beef burger–bun combination may be due to analytical inaccuracies for tryptophan resulting in very high measured values.

A few values for SID were above 100%, which is not biologically possible. Basal endogenous losses were determined using an N-free diet, which many have slightly overestimated ileal endogenous losses and thereby resulted in SID values above 100%. However, the SID of most AA in ingredients based on the nitrogen-free diet method is additive in mixed diets, and the use of a nitrogen-free diet is preferred over purified proteins to measure endogenous basal loss of AA because of its simplicity and the definition of basal endogenous loss of AA [11, 37]. It has also been demonstrated that although at least 3 days of adaptation is required, there is no differences in basal endogenous losses measured on d 6 and 7 or on d 8 and 9 [38].

Results for DIAAS in the beef burgers are close to published values for DIAAS in processed bovine meat [26], and the observation that DIAAS was greater than 100 in the pork burger is also in agreement with published values for DIAAS in pork products [25]. Likewise, the fact that DIAAS was less than 100 in the burger bun and in the plant burgers is in agreement with published DIAAS values for cereal grains [19, 21] and other plant-based proteins [23]. The observation

Table 7 Measured and predicted values for apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein and amino acids (AA) in the Impossible Burger and bun meal

	AID				SID			
Item, %	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE
Crude protein	81.6	81.1	0.5	2.60	98.0	97.8	0.2	2.60
Indispensable AA								
Histidine	86.5	87.9	− 1.4	1.87	92.9	94.6	− 1.7	1.87
Isoleucine	86.8	88.1	− 1.3	1.64	91.8	93.3	− 1.4	1.64
Leucine	87.8	88.8	− 1.0	1.69	92.4	93.7	− 1.2	1.69
Lysine	84.2	81.8	2.4	2.03	91.8	90.1	1.7	2.03
Methionine	88.1	89.4	− 1.2	1.45	92.5	94.0	− 1.5	1.45
Phenylalanine	89.5	90.4	− 0.9	1.43	93.6	94.6	− 1.1	1.43
Threonine	75.2	77.7	− 2.5	3.21	86.7	89.7	− 3.0	3.21
Tryptophan	92.0	91.3	0.7	1.92	98.1	97.6	0.5	1.92
Valine	84.7	86.0	− 1.3	1.99	91.0	92.5	− 1.6	1.99
Mean	85.8	86.7	− 0.9	1.89	91.9	93.0	− 1.2	1.89
Dispensable AA								
Alanine	81.6	80.3	1.3	2.34	95.2	94.3	0.8	2.34
Arginine	89.4	87.5	1.9	1.54	103.3	102.3	1.0	1.55
Asx	79.2	80.6	− 1.4	2.41	86.0	87.7	− 1.7	2.41
Cystein	71.3	75.8	− 4.5	2.83	77.0	82.0	− 4.9	2.83
Glx	93.3	93.8	− 0.5	0.82	96.0	96.7	− 0.7	0.82
Glycine	70.1	65.8	4.2	3.97	108.5	106.5	2.0	3.97
Serine	83.1	83.9	− 0.8	2.13	92.3	93.7	− 1.4	2.13
Tyrosine	86.4	86.3	0.2	1.88	92.8	93.6	− 0.8	1.88
Mean	86.4	86.4	0.0	1.61	94.8	95.3	− 0.6	1.61
Total AA	86.2	86.5	− 0.3	1.73	93.7	94.4	− 0.7	1.73

Asx sum of asparagine and aspartic acid, *Glx* sum of glutamine and glutamic acid, *SE* standard error

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

that the animal-based burgers had DIAAS values greater than 100 with no limiting AA for both age groups, indicates that the animal burgers are "excellent" sources of protein [7]. However, because the Impossible Burger had DIAAS greater than 75 and less than 100 for children less than 3 years old, this burger only qualifies as a "good" source of protein according to the Food and Agricultural Organization [7]. In contrast, because the Impossible Burger had DIAAS greater than 100 for individuals older than 3 years, it is an "excellent" source of protein for this age group [7]. For children older than 3 years, adolescents, and adults, the Beyond Burger qualifies as a "good" source of protein [7], but due to the low DIAAS in Beyond Burger for children from 6 months to 3 years, no protein claim can be made for this age group [7].

The Impossible Burger was produced using soy protein concentrate as the main source of protein with coconut oil and sunflower oil being the sources of oil. The Beyond Burger was based on pea protein isolate as the main source of protein, whereas expeller-pressed canola oil and refined coconut oil were the sources of oil. It is, therefore, not surprising that DIAAS for the Impossible Burger was greater than that of the Beyond Burger, because soy protein concentrate has a greater DIAAS than pea protein isolate [23].

The observation that the animal-based burger and bun combinations had DIAAS very close to or equal to 100 for children from 6 months to 3 years, and greater than 100 for individuals older than 3 years, indicates that the high protein quality in animal meat can compensate for the low protein quality in the burger bun. As a consequence, the combination of animal burger and bun provides a balanced ratio of indispensable AA. The low DIAAS in the combination of Impossible Burger and bun for both age groups, and with lysine as first limiting AA, demonstrates that there is insufficient lysine in the Impossible Burger to compensate for the low concentration of lysine in the burger bun. The ingredients used to produce the burger bun were grain-based ingredients, and cereal grains always have lysine as first limiting AA [19, 21]. In addition to lysine, leucine, SAA, and threonine, were also limiting in the Impossible Burger–bun combination for children from 6 months to 3 years, but for individuals older than 3 years, only lysine was limiting.

Table 8 Digestible indispensable amino acids (DIAA) reference ratio and digestible indispensable amino acid score (DIAAS) in food ingredients as measured in growing pigs

Item	80% lean beef	93% lean beef	Pork	Impossible Burger	Beyond Burger	Burger bun	SE	*P* value
Child (6 months to 3 years)[†]								
DIAA reference ratio								
Histidine	1.27	1.47	1.55	1.12	1.11	0.92		
Isoleucine	1.24	1.45	1.38	1.41	1.40	1.06		
Leucine	1.03	1.16	1.11	1.06	1.14	0.91		
Lysine	1.25	1.44	1.36	0.95	1.11	0.26		
SAA	1.03	1.22	1.17	0.91	0.71	1.21		
AAA	1.28	1.42	1.39	1.62	1.69	1.35		
Threonine	1.10	1.30	1.29	1.09	1.02	0.75		
Tryptophan	1.16	1.41	1.42	1.45	1.05	1.20		
Valine	1.02	1.11	1.11	1.13	1.14	0.87		
DIAAS[¥], %	102^{b}	111^{a}	111^{a}	91^{c} (SAA)	71^{d} (SAA)	26^{e} (Lysine)	2.36	< 0.001
Older child, adolescent, adult[†]								
DIAA reference ratio								
Histidine	1.58	1.84	1.94	1.40	1.39	1.15		
Isoleucine	1.33	1.54	1.47	1.51	1.49	1.13		
Leucine	1.11	1.25	1.20	1.14	1.23	0.98		
Lysine	1.49	1.71	1.62	1.13	1.32	0.31		
SAA	1.20	1.43	1.38	1.07	0.83	1.42		
AAA	1.62	1.81	1.77	2.06	2.15	1.72		
Threonine	1.36	1.62	1.60	1.35	1.27	0.93		
Tryptophan	1.49	1.81	1.83	1.87	1.35	1.54		
Valine	1.10	1.19	1.19	1.21	1.23	0.93		
DIAAS[¥], %	110^{b}	119^{a}	119^{a}	107^{b}	83^{c} (SAA)	31^{d} (Lysine)	2.51	< 0.001

AAA aromatic amino acid, *SAA* sulfur amino acid, *SE* standard error

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

[†] DIAA reference ratios and DIAAS were calculated using the recommended indispensable amino acids scoring pattern, expressed as mg amino acid/g protein [7]. Child: Histidine 20, Isoleucine 32, Leucine 66, Lysine 57, SAA 27, AAA 52, Threonine 31, Tryptophan 8.5, and Valine 43 [7]. Older child, adolescent and adult: Histidine 16, Isoleucine 30, Leucine 61, Lysine 48, SAA 23, AAA 41, Threonine 25, Tryptophan 6.6, and Valine 40 [7]

[¥] First-limiting AA in parentheses

Thus, to meet AA requirements for children from 6 months to 3 years, the Impossible Burger–bun combination needs to be complemented by another high lysine ingredient that also provides some leucine, SAA, and Threonine.

With the exception of the tendency for a greater predicted DIAAS in the pork burger–bun combination compared with the measured DIAAS for individuals older than 3 years, no differences between the measured and the predicted DIAAS were observed. This demonstrates that by measuring DIAAS in individual food ingredients, DIAAS in combined meals can be predicted with reasonable accuracy. This is an important observation, because it will not be possible to measure DIAAS in all possible combinations of foods. However, if DIAAS is measured in individual ingredients, the protein quality of mixed meals or combinations of foods can be predicted as demonstrated in this experiment. This is in agreement with recent data demonstrating that DIAAS in milk and breakfast cereals can be used to accurately predict DIAAS in breakfast cereal–milk combinations [8].

Ideally, humans should be used to determine DIAAS in human foods [11]. However, collecting digesta from the distal ileum in humans is invasive and expensive, and although methods that do not require digesta sampling have been developed, they are not suitable for routine food evaluation [10, 11]. As a result, the growing pig, is recommended for determining DIAAS in human foods [7]. The advantage of using a pig model over clinical trials is that it is a rapid method and there is already a large dataset with values for SID of AA in foods that can be translated to the human dataset with a subsequent calculation of DIAAS [10]. Other benefits of this model include the ability to determine effects of processing on DIAAS [8, 10], and there are a number of

Table 9 Measured and predicted values for digestible indispensable amino acids (DIAA) reference ratio and digestible indispensable amino acid score (DIAAS) in combined meals of burgers and bun

Item	80% lean beef burger + bun				Pork burger + bun				Impossible burger + bun			
	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE	Measured	Predicted	Difference	SE
Child (6 months to 3 years)†												
DIAA reference ratio												
Histidine	1.18	1.17	0.01	0.02	1.31	1.35	– 0.04^{+}	0.02	1.03	1.05	– 0.02	0.02
Isoleucine	1.19	1.19	0.00	0.02	1.24	1.28	– 0.03^{+}	0.01	1.27	1.29	– 0.02	0.02
Leucine	1.00	0.99	0.00	0.01	1.02	1.04	– 0.02	0.01	0.99	1.01	– 0.01	0.02
Lysine	0.99	0.97	0.02	0.02	1.00	1.00	0.00	0.02	0.73	0.71	0.01	0.02
SAA	1.08	1.08	0.01	0.02	1.14	1.19	– 0.05*	0.01	0.97	1.02	– 0.04	0.03
AAA	1.31	1.30	0.02	0.02	1.36	1.38	– 0.02	0.02	1.51	1.53	– 0.02	0.03
Threonine	1.01	1.00	0.01	0.03	1.06	1.12	– 0.05^{+}	0.02	0.94	0.97	– 0.03	0.04
Tryptophan	1.26	1.17	0.09**	0.02	1.34	1.35	0.00	0.02	1.37	1.36	0.01	0.03
Valine	0.98	0.98	0.00	0.02	1.00	1.03	– 0.03^{+}	0.01	1.02	1.04	– 0.02	0.02
DIAAS¥, %	98 (Valine)	97 (Lysine)	– 0.17	1.86	100	100	– 0.83	1.59	73 (Lysine)	71 (Lysine)	1.38	1.62
Older child, adolescent, adult†												
DIAA reference ratio												
Histidine	1.47	1.46	0.01	0.03	1.63	1.68	– 0.05^{+}	0.02	1.29	1.31	– 0.02	0.03
Isoleucine	1.27	1.27	0.00	0.02	1.33	1.36	– 0.03^{+}	0.02	1.36	1.38	– 0.02	0.02
Leucine	1.08	1.07	0.00	0.01	1.10	1.13	– 0.02	0.01	1.07	1.09	– 0.02	0.02
Lysine	1.17	1.16	0.02	0.02	1.19	1.19	0.00	0.02	0.86	0.85	0.02	0.02
SAA	1.27	1.27	0.01	0.03	1.34	1.39	– 0.05*	0.02	1.14	1.19	– 0.05	0.08
AAA	1.66	1.65	0.02	0.02	1.72	1.75	– 0.03	0.02	1.92	1.94	– 0.02	0.03
Threonine	1.26	1.24	0.01	0.04	1.32	1.38	– 0.06^{+}	0.03	1.16	1.20	– 0.04	0.04
Tryptophan	1.63	1.51	0.12**	0.02	1.73	1.73	0.00	0.03	1.76	1.75	0.01	0.03
Valine	1.05	1.05	0.00	0.02	1.07	1.11	– 0.03^{+}	0.01	1.10	1.12	– 0.02	0.02
DIAAS¥, %	105	105	0.18	1.91	107	111	– 3.17^{+}	1.53	86 (Lysine)	85 (Lysine)	1.62	1.93

AAA aromatic amino acid, *SAA* sulfur amino acid, *SE* standard error

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

†DIAA reference ratios and DIAAS were calculated using the recommended indispensable amino acids scoring pattern, expressed as mg amino acid/g protein [7]. Child: Histidine 20, Isoleucine 32, Leucine 66, Lysine 57, SAA 27, AAA 52, Threonine 31, Tryptophan 8.5, and Valine 43 [7]. Older child, adolescent and adult: Histidine 16, Isoleucine 30, Leucine 61, Lysine 48, SAA 23, AAA 41, Threonine 25, Tryptophan 6.6, and Valine 40 [7]

¥First-limiting AA in parentheses

laboratories around the world that are capable of determining digestibility of AA by pigs [11]. However, one of the limitations of using an animal model is that, despite well-described similarities in AA digestibility between growing pigs and humans, protein digestibility and nutrient absorption are unlikely to be identical, and data from pigs do not account for effects of different social and environmental conditions [11].

Conclusion

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

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Author contributions All authors contributed to the study conception and design. MNN, RD, and TT prepared the burgers used in the experiment. NSF conducted the animal part of the research and analyzed the data. HMB assisted with statistical analyses. MNN, RD, and TT contributed to the interpretation of the data. NSF wrote the first draft of the manuscript. HHS supervised the project and had primary responsibility for the final content. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Availability of data and materials All data generated or analyzed during this research are included in this manuscript.

Code availability Not applicable.

Ethics approval The study was approved by the Institutional Animal Care and Use Committee at the University of Illinois under the United States Department of Agriculture Animal Welfare Act and the NIH Public Health Service Policy on the Humane Care and Use of Animals; Protocol license number: 19131.

Consent to participate/for publication Not applicable—the manuscript does not contain clinical studies or patient data.

References

1. Daniel CR, Cross AJ, Koebnick C, Sinha R (2011) Trends in meat consumption in the USA. Public Health Nutr 14:575–583. https://doi.org/10.1017/S1368980010002077
2. Speedy AW (2003) Global production and consumption of animal source foods. J Nutr 133:4048S-4053S. https://doi.org/10.1093/jn/133.11.4048S
3. FAOSTAT (2018) Food supply—crops primary equivalent. http://www.fao.org/faostat/en/#data/CC. Accessed 30 Apr 2021
4. Björn W, Przemek O, Sedef K, Benjamin M, Michael B, Jürgen R, Ulrik S, Decker W, Elfrun VK, Nico D, Friederike GH (2021) Food for thought: the protein transformation. Boston Consulting Group. https://www.bcg.com/publications/2021/the-benefits-of-plant-based-meats. Accessed 30 Apr 2021
5. Ruby MB (2012) Vegetarianism. A blossoming field of study. Appetite 58:141–150. https://doi.org/10.1016/j.appet.2011.09.019
6. Beardsworth AD, Keil ET (1991) Vegetarianism, veganism, and meat avoidance: recent trends and findings. Br J Nutr 93:19–24. https://doi.org/10.1108/00070709110135231
7. Food and Agriculture Organization of the United Nations—FAO (2013) Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation, Rome
8. Fanelli NS, Bailey HM, Guardiola LV, Stein HH (2021) Values for digestible indispensable amino acid score (DIAAS) determined in pigs are greater for milk than for breakfast cereals, but DIAAS values for individual ingredients are additive in combined meals. J Nutr 151:540–547. https://doi.org/10.1093/jn/nxaa398
9. United States Department of Agriculture and Food Safety and Inspection Service (2016) Meat preparation. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation. Accessed 22 Aug 2019
10. Hodgkinson SM, Stein HH, de Vries S, Hendriks WH, Moughan PJ (2020) Determination of true ileal amino acid digestibility in the growing pig for calculation of digestible indispensable amino acid score (DIAAS). J Nutr 150:2621–2623. https://doi.org/10.1093/jn/nxaa210
11. Food and Agriculture Organization of the United Nations – FAO (2014) Research approaches and methods for evaluating the protein quality of human foods. Report of an FAO expert working group, Rome
12. National Research Council—NRC (2012) Nutrient requirements of swine. 11th rev edition. Washington, DC
13. Stein HH, Shipley CF, Easter RA (1998) Technical note: a technique for inserting a T-cannula into the distal ileum of pregnant sows. J Anim Sci 76:1433–1436. https://doi.org/10.2527/1998.7651433x

14. AOAC International (2007) Official methods of analysis of AOAC International, 18th ed. Rev. 2. Gaithersburg (MD)
15. US Environmental Protection Agency (2000) Acid digestion of sediments sludges and soils US. EPA, Washington, DC
16. Myers WD, Ludden PA, Nayigihugu V, Hess BW (2004) Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. J Anim Sci 82:179–183. https://doi.org/10.2527/2004.821179x
17. Stein HH, Sève B, Fuller MF, Moughan PJ, de Lange CFM (2007) Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J Anim Sci 85:172–180. https://doi.org/10.2527/jas.2005-74
18. Stein HH, Pedersen C, Wirt AR, Bohlke RA (2005) Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J Anim Sci 83:2387–2395. https://doi.org/10.2527/2005.83102387x
19. Han F, Han F, Wang Y, Fan L, Song G, Chen X, Jiang P, Miao H, Han Y (2019) Digestible indispensable amino acid scores of nine cooked cereal grains. Br J Nutr 121:30–41. https://doi.org/10.1017/S0007114518003033
20. Abelilla JJ, Liu Y, Stein HH (2018) Digestible indispensable amino acid score (DIAAS) and protein digestibility corrected amino acid score (PDCAAS) in oat protein concentrate measured in 20-to 30-kilogram pigs. J Sci Food Agric 98:410–414. https://doi.org/10.1002/jsfa.8457
21. Cervantes-Pahm SK, Liu Y, Stein HH (2014) Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. Br J Nutr 111:1663–1672. https://doi.org/10.1017/S0007114513002427
22. Rutherfurd SM, Fanning AC, Miller BJ, Moughan PJ (2015) Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. J Nutr 145:372–379. https://doi.org/10.3945/jn.114.195438
23. Mathai JK, Liu Y, Stein HH (2017) Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). Br J Nutr 117:490–499. https://doi.org/10.1017/S0007114517000125
24. Bailey HM, Stein HH (2020) Raw and roasted pistachio nuts (*Pistacia vera* L.) are 'good'sources of protein based on their digestible indispensable amino acid score as determined in pigs. J Sci Food Agric 100:3878–3885. https://doi.org/10.1002/jsfa.10429
25. Bailey HM, Mathai JK, Berg EP, Stein HH (2020) Pork products have digestible indispensable amino acid scores (DIAAS) that are greater than 100 when determined in pigs, but processing does not always increase DIAAS. J Nutr 150:475–482. https://doi.org/10.1093/jn/nxz284
26. Hodgkinson SM, Montoya CA, Scholten PT, Rutherfurd SM, Moughan PJ (2018) Cooking conditions affect the true ileal digestible amino acid content and digestible indispensable amino acid score (DIAAS) of bovine meat as determined in pigs. J Nutr 148:1564–1569. https://doi.org/10.1093/jn/nxy153
27. Bailey HM, Mathai JK, Berg EP, Stein HH (2020) Most meat products have digestible indispensable amino acid scores that are greater than 100, but processing may increase or reduce protein quality. Br J Nutr 24:14–22. https://doi.org/10.1017/S0007114520000641
28. United States Department of Agriculture Agricultural Research Service (2019) USDA food composition database. https://ndb.nal.usda.gov. Accessed 14 Dec 2020
29. United States Department of Agriculture Agricultural Research Service (2020) USDA food composition database. https://ndb.nal.usda.gov. Accessed 14 Dec 2020
30. Jaeger H, Janositz A, Knorr D (2010) The Maillard reaction and its control during food processing. The potential of emerging technologies. Pathol Biol 58:207–213. https://doi.org/10.1016/j.patbio.2009.09.016
31. Almeida FN, Htoo JK, Thomson J, Stein HH (2014) Effects of heat treatment on apparent and standardized ileal digestibility of amino acids in canola meal fed to growing pigs. Anim Feed Sci Technol 187:44–52. https://doi.org/10.1016/j.anifeedsci.2013.09.009
32. González-Vega JC, Kim BG, Htoo JK, Lemme A, Stein HH (2011) Amino acid digestibility in heated soybean meal fed to growing pigs. J Anim Sci 89:3617–3625. https://doi.org/10.2527/jas.2010-3465
33. Stein HH, Trottier NL, Bellaver C, Easter RA (1999) The effect of feeding level and physiological status on total flow and amino acid composition of endogenous protein at the distal ileum in swine. J Anim Sci 77:1180–1187. https://doi.org/10.2527/1999.7751180x
34. Almeida FN, Petersen GI, Stein HH (2011) Digestibility of amino acids in corn, corn co-products and bakery meal fed to growing pigs. J Anim Sci 89:4109–4115. https://doi.org/10.2527/jas.2011-4143
35. Casas GA, Almeida JAS, Stein HH (2015) Amino acid digestibility in rice co-products fed to growing pigs. Anim Feed Sci Technol 207:150–158. https://doi.org/10.1016/j.anifeedsci.2015.05.024
36. Xue PC, Ragland D, Adeola O (2014) Determination of additivity of apparent and standardized ileal digestibility of amino acids in diets containing multiple protein sources fed to growing pigs. J Anim Sci 92:3937–3944. https://doi.org/10.2527/jas.2014-7815
37. Adeola O, Xue PC, Cowieson AJ, Ajuwon KM (2016) Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim Feed Sci Technol 221:274–283. https://doi.org/10.1016/j.anifeedsci.2016.06.004
38. Kim BG, Lee SA, Park KR, Stein HH (2020) At least three days of adaptation are required before indigestible markers (chromium, titanium, and acid insoluble ash) are stabilized in the ileal digesta of 60-kg pigs, but values for amino acid digestibility are affected by the marker. J Anim Sci 98:1–8. https://doi.org/10.1093/jas/skaa027