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Comparison of True Ileal Amino Acid Digestibility between Adult Humans and Growing Pigs

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ABSTRACT

Background: It is not feasible to determine the true ileal amino acid (AA) digestibility of protein sources in humans on a routine basis, and the growing pig has been recommended as an animal model for this purpose but requires further validation.

Objectives: To determine and compare true ileal AA digestibility between adult human ileostomates and growing cannulated pigs for a range of food proteins.

Methods: Seven protein sources (black beans, bread, collagen, pigeon peas, wheat bran, whey protein isolate, and zein) that spanned the range of digestibilities typically seen in foods were evaluated. Six female growing pigs received each of the protein sources, as well as a protein-free diet, and digesta were collected via ileal T-cannula. Adult human ileostomates consumed the same protein sources (5–8 ileostomates, depending on the protein source), as well as a protein-free diet, and digesta were collected. Titanium dioxide and celite were included in the diets as indigestible markers. True ileal AA digestibility coefficients were determined.

Results: There was a significant effect of protein source ($P \leq 0.001$) for all AAs. The effect of species was not significant ($P > 0.05$) except for total lysine (but not for available lysine). When analyzed within diets, the statistically significant species effect for true lysine digestibility was found for black beans only. Pig and human digestibility values were generally highly and significantly ($P \leq 0.05$) correlated. A linear regression equation derived for true ileal AA digestibility (given as coefficients) determined in the human and pig for the overall mean of all AAs was (y = human, x = pig) y = 1.00x – 0.010, with the slope not statistically significant ($P > 0.05$) from unity and the intercept not different ($P > 0.05$) from zero.

Conclusions: True ileal AA digestibility values determined in the growing pig can be directly used for predicting digestibility in adult humans. *J Nutr* 2022;152:1635–1646.

Keywords: true ileal amino acid digestibility, human ileostomates, cannulated pig, DIAAS, protein quality

Introduction

In 2013, an FAO Expert Consultation recommended the use of a digestible indispensable amino acid score (DIAAS) for evaluating protein quality of foods and food ingredients for humans (1). The calculation of DIAAS requires data on the content of true (corrected for gut nondietary amino acids) ileal (determined at the end of the small intestine) digestible amino acids (AAs) of the food and data on true ileal digested structurally unaltered (reactive) lysine. Particularly in animal nutrition, true ileal AA digestibility is often referred to as "standardized" ileal AA digestibility. When used for regulatory purposes, DIAAS is determined by comparing concentrations of true ileal digestible indispensable AAs, one-on-one, to recommended AA requirements for a child (aged 6 mo to 3 y), expressed as amounts of AAs per gram of protein (1). The first limiting AA defines the DIAAS.

When calculating DIAAS, ideally the true ileal AA digestibility values would be determined directly in humans. To do so, however, requires the collection of digesta from the terminal

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Abbreviations used: AA, amino acid; AIA, acid insoluble ash marker; DIAAS, digestible indispensable amino acid score; DM, dry matter; IAAO, indicator amino acid oxidation; Ti, titanium; TiO_2, titanium dioxide.

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ileum, which is not straightforward. One option is the use of nasoileal intubation (2), which involves passing a small-caliber tube through the nose, down the back of the throat, and then along the digestive tract to the terminal ileum. Digesta can then be aspirated through the tube after the protein source has been consumed. This method, however, although providing valuable data in humans with normal digestive tracts, is invasive (3), relies on multiple markers, and has the limitation that, due to the small-caliber tube that is required, the method can only be applied if the particle size of the digesta is sufficiently fine. Less invasive indirect methods to determine AA digestibility or availability in humans, such as the dual-isotope method (4) and indicator AA oxidation (IAAO) method (5), are also options. However, these methods, although showing much promise, cannot be considered routine methods and either are still under development or have not been fully validated (3, 6). With the IAAO method, relatively costly intrinsic labeling of the food is not required, which is the case for the dual-isotope approach, but AA availability observations are limited to one AA per assay period, which requires multiple testing days.

An alternative means of directly determining AA digestibility via sampling ileal digesta is afforded by the cooperation of human ileostomates. These are people who have had their large intestine removed for medical reasons and their terminal ileum exteriorized. They can consume the protein source to be tested, and digesta can then be collected and true ileal AA digestibility of the protein source determined (7). Protein sources that can be evaluated with this method are not limited by particle size, and the method allows measurement of the entire AA profile of the ingested proteins.

It is not practically feasible, however, to determine the true ileal AA digestibility of protein sources with the cooperation of ileostomates on a routine basis for large numbers of protein sources. For this purpose, it is necessary to use an animal model, and the growing pig has been recommended for this purpose (1). It is important, however, to demonstrate that the results obtained with the animal model are valid for use in human nutrition.

Similar true ileal AA digestibility coefficients have been found in the growing pig and adult human (2, 8), but in these studies, in the main, highly digestible foods were evaluated (9), although in the study by Deglaire et al. (2), there was also good agreement between the growing pig and adult human for true ileal nitrogen digestibility for a less digestible rapeseed protein. Although the results from these controlled studies support the growing pig cannulated at the terminal ileum as a model for protein digestibility in the adult human, the FAO Expert Consultation (1) considered it necessary to have a wider evidence base and called for a further pig/human AA digestibility comparison. The FAO Expert Consultation envisaged the development of a robust regression relation between human true ileal AA digestibility (adult human ileostomates) and pig true ileal AA digestibility (T-cannulated growing pig) to allow establishment of a large databank of predicted human true ileal AA digestibility values for human foods fed to pigs in the same form as they are consumed by humans. The growing pig would be used as the preferred animal model for the adult human for more routine determination of true ileal AA digestibility. The pig has the advantage of being a meal-eating omnivore and eating most foods consumed by humans.

The study described here responds to the FAO Expert Consultation call (1), with the aim being to determine and compare true ileal AA digestibility between adult human ileostomates and growing cannulated pigs for a wide range of food protein sources.

Methods

Ethical approval

The study with humans was conducted in New Zealand and the Netherlands. The New Zealand (Riddet Institute) study was conducted in 2 parts. The first Riddet Institute study with human ileostomates was approved by the Massey University Human Ethics Committee Southern A (ref. 16/59), as well as the New Zealand Ministry of Health Central Health and Disability Ethics Committee (ref. 18/CEN/215). The second Riddet Institute study with ileostomates was approved by the Massey University Human Ethics Committee Southern A (ref. 20/08). The studies were registered in the Australian New Zealand Clinical Trials Registry (ACTRN: 12618001518257). The Dutch experimental protocol for the human study was approved by the medical ethical committee of Wageningen University (NL63446.081.17), and the clinical trial reference was NCT04207372. A single study with growing pigs was undertaken in New Zealand, and the study was approved by the Massey University Animal Ethics Committee (protocol number 16/121).

Selection and preparation of protein sources

Seven protein sources were selected that spanned the range of digestibilities typically seen in foods (the determined true ileal mean AA digestibility values ranged from 60% to 98%). A mixture of different "types" of protein (beans, cereals, meat product, dairy product, processed foods, purified protein) was included. The list of protein sources and their AA composition is given in **Table 1**. All protein sources and ingredients were food grade and procured directly from commercial sources, with the exception of the wheat bread. All protein sources were stored according to manufacturers' instructions and were fed to humans or pigs before their expiry dates.

The preparation of each protein source was the same for the human and pig studies. This information is given in the **Supplemental Materials**.

Pig studies

For the pig studies, each protein source, after its preparation as described in Supplemental Materials, was combined immediately before feeding with a specific mixture of nonprotein food ingredients. This mixture was specific for each food source but generally included purified maize starch (at a level to give a final protein concentration of 100 g/kg in each diet), sucrose, vitamins and minerals, refined vegetable oil, and purified cellulose. Titanium dioxide (TiO_2) and celite were also included in the nonprotein mixture to serve as indigestible markers. The diet compositions for the test diets are given in **Supplemental Table 1**. Basal and protein-free diets were also prepared (**Supplemental Table 2**). The basal diet was formulated to be structurally similar to a human diet and was fed to the animals during the presurgery acclimatization period, during the recovery period between surgery and the assay period, and for 7 d following the feeding of the protein-free diet.

The feeding/digesta collection method used was that described in detail by Hodgkinson et al. (10) and follows the recommendations of an FAO Working Group (4). Twelve female pigs (Landrace/Large White) were used. The pigs weighed 30.5 ± 0.30 kg at the beginning of the study. The animals were housed individually in smooth-sided pens (1.5 × 1.5 m) with slatted floors. The room was kept at 21–24°C (thermoneutral zone), with a 12-h light/dark cycle. Fresh water was freely available to all animals at all times.

Throughout the study, the daily dietary ration for each pig was 0.08 × metabolic bodyweight ($kg^{0.75}$) calculated on a dry matter (DM) basis. The daily ration was given in 2 equal meals 9 h apart (08:00 and 17:00). Pigs were weighed weekly (each time the diet was changed), and the daily ration of each pig was adjusted according to the bodyweight of the pig.

TABLE 1 Protein and amino acid composition of the protein sources

	Dry matter, mg/g						
Characteristic	Black beans[1]	Bovine collagen[2]	Pigeon peas[3]	Toasted wheat bread[4]	Wheat bran[5]	Whey protein isolate[6]	Zein[7]
Protein	265	1000	238	99	138	863	919
Histidine	5.02	7.28	8.12	2.44	2.38	13.2	10.1
Isoleucine	8.29	15.2	9.80	3.95	2.99	58.7	29.0
Leucine	15.2	27.7	17.4	7.78	6.26	89.7	156.0
Reactive lysine	11.3	36.0	16.7	1.38	1.48	82.7	0.00
Total lysine	13.2	36.0	17.0	2.08	1.87	82.8	0.00
Methionine	2.16	8.45	1.84	1.81	1.56	20.7	13.5
Phenylalanine	11.1	19.9	12.2	5.56	4.29	25.7	52.1
Tyrosine	6.90	7.47	8.04	3.84	2.96	24.5	40.5
Threonine	7.61	15.2	8.48	3.14	2.60	60.5	22.1
Tryptophan	2.20	1.53	1.90	1.35	1.48	13.3	0.00
Valine	9.41	23.2	11.5	4.81	4.29	52.6	30.3
Alanine	7.06	82.4	9.45	3.37	3.71	44.1	82.8
Arginine	13.0	81.3	19.9	4.10	5.96	17.2	12.7
Aspartic acid	18.2	30.2	25.9	4.40	3.99	91.3	46.3
Glutamic acid	28.4	78.7	38.9	38.0	25.3	158.5	219.2
Serine	10.3	22.2	12.0	5.37	4.15	39.6	45.6

[1] Harvest North.
[2] Dat-Schaub.
[3] Davis Food Ingredients.
[4] See Supplemental Materials.
[5] Kellogg's All Bran (Aust.) Pty. Ltd.
[6] Fonterra.
[7] Sigma.

After the pigs were adapted to the environment and basal diet for 8 d, a titanium T-cannula was surgically inserted into the end of the small intestine (terminal ileum) of each pig using the procedure described in detail by Hodgkinson et al. (10). The pigs were then given at least 8 d to recover from surgery prior to starting the assay phase.

Six pigs received each experimental diet, with each pig receiving 3 or 4 protein sources in total. Test cycles for each test food had a 7-d duration. Pigs were allotted to their test cycles according to an incomplete Latin square (Youden square), with diets and periods comprising the rows and the columns of the squares, respectively.

The initial 5 d of each test were the adaptation period to the diet. Digesta were collected from the cannula for 9 h on days 6 and 7 of each test cycle starting immediately after the first meal of the day, via small plastic bags attached to the cannula barrel using an elastic band. The bags contained 2 mL of 0.2 mol/L HCl as an antimicrobial agent. Bags were replaced whenever filled with digesta and at least once every 30 min, and the digesta were immediately frozen (–20°C).

Each pig also received a protein-free diet for 7 d to allow for the correction for endogenous (nondietary) AA excretions on an individual animal basis. The pig was its own control for the correction of endogenous AAs. The pigs received the protein-free diet after they had received 3 protein sources (after 3 test cycles). The feeding of the protein-free diet and subsequent collection of ileal digesta were carried out in the same manner as for the test diets. Following the protein-free diet, the basal diet was fed to the pigs for a period of 7 d, before beginning the following test cycle, to minimize carryover effects from the protein-free diet.

Human studies

Supplemental Figures 1 and **2** present flowcharts describing the studies involving human ileostomates.

Study 1.

The protein sources whey protein isolate (WPI) and zein were evaluated with adult human ileostomates at Wageningen UR, the Netherlands. Adult ileostomates at the Riddet Institute, New Zealand, received 5 protein sources. The same methods were used in all cases, and the protein sources that the humans received were the same material (same batch) and prepared in the same manner as for the pigs.

For the study carried out at Wageningen UR, males and females were recruited via advertisement by Hospital de Gelderse Vallei (Ede, the Netherlands) and the Dutch Ostomy Association. For the study carried out at the Riddet Institute, potential participants were identified by staff at the Palmerston North Hospital and sent an invitation to participate. In total, 10 ileostomates were recruited by Wageningen UR with 8 completing the study, and 6 ileostomates were recruited by the Riddet Institute. Inclusion criteria for the study included having a fully functional conventional ileostomy, age 18–70 y, BMI (in kg/m^2) between 18 and 28, and no more than 20 cm of the small intestine removed in prior surgery.

Exclusion criteria included being pregnant or breastfeeding in the past 12 mo, smoking cigarettes, recreational drug use, high level of alcohol consumption, renal impairment or diabetes, use of antibiotics or other medications that affect small intestinal digestion or absorption, being vegetarian or vegan, or having an allergy to dairy products, a gluten intolerance, or an allergy to proteins.

The participants at Wageningen UR had a total of 6 study days, whereas those at the Riddet Institute had 12 study days: 2 study days per protein source and another 2 study days for the protein-free diet. The study days were always separated by at least 3 d. On each study day, the participants received a meal that contained the test protein as the sole protein source or the protein-free diet following a 14- to 15-h overnight fast. The meal was consumed within 30 min. Participants received 25 g of protein in the meal, as described by Moughan et al. (7). For several of the protein sources, the participants also received protein-free biscuits (purified corn flour, margarine, sucrose, cellulose, baking powder, ginger; refer to Supplemental Materials) prepared as described by Moughan et al. (7). The biscuits contained celite at the same concentration as the protein source on a DM basis. The preparation of the protein sources immediately prior to being consumed by the human participants is described in the Supplemental Materials, and the amounts of each ingredient in the meals for all human studies are shown in **Supplemental Tables 3** and **4**.

A new ostomy bag was attached just before consuming the test meal. After the participants had consumed their test meal, ileal digesta were collected through the ostomy bags for the following 9 h. The bags were emptied into 1 of 2 containers on ice (first container for first half of collection period, second container for second half of collection period), which contained 5 mL of 4 M HCl, at least every 2 h. During this 9-h period, the participants only consumed, at their own discretion, sweetened drinks (no protein), herbal teas (not containing polyphenols) or coffee (with no milk), or energy drinks devoid of protein. The containers containing digesta were frozen (–20°C) upon completion of the sampling period.

Study 2.

Because of concerns regarding recovery of the acid insoluble ash marker (AIA) for some diets during the first study (see below), the study was repeated at the Riddet Institute for some protein sources, with TiO_2 (0.2%) being included as the indigestible marker. Only 3 of the participants from study 1 were prepared to continue in study 2. For this reason, only the protein sources black beans, pigeon peas, and wheat bran were included, along with the protein-free diet. The method used was the same as that described for human study 1, and the ingredient compositions of the meals are presented in Supplemental Table 4.

Chemical analysis

Digesta were thawed but maintained at <4°C. After pooling and mixing the digesta, a subsample of the digesta from each pig and diet was collected and freeze-dried. The test foods, test diets, and digesta samples were sampled using standard sampling procedures. The following chemical analyses were carried out: DM according to the method described by AOAC International (11), titanium following the method of Short et al. (12), and AIA according to McCarthy (13). The AA contents of the test foods, test diets, and ileal digesta samples were determined using the methods described by Rutherfurd et al. (14, 15) involving a 24-h acid hydrolysis. The tryptophan contents of test foods, test diets, and ileal digesta samples were determined using alkaline hydrolysis. The weight of each AA was calculated using free AA molecular weights, and no correction was made for potential destruction/further release of AAs during the 24-h hydrolysis. The reactive (structurally altered) lysine content of the food and digesta samples was determined as described by Moughan and Rutherfurd (16).

Calculations

Values for basal gut endogenous AAs were those determined for each human or pig fed the protein-free diet. The values determined for each human or pig were used to calculate true ileal digestibility coefficients on an individual basis (i.e., each human or pig was its own control for the correction of endogenous AAs).

Calculations for pig data.

Equations 1 and 2 were used to calculate the true ileal AA digestibility coefficients for the protein sources when fed to the pig using TiO_2 or AIA as the marker.

$$\text{Ileal AA } (\mu g/g\,\text{DMI}) = \frac{\text{Concentration of AA in digesta } (\mu g/g\,\text{DM}) \times \text{Diet marker concentration } (\mu g/g\,\text{DM})}{\text{Digesta marker concentration } (\mu g/g\,\text{DM})} \quad (1)$$

True ileal AA digestibilities were then calculated:

$$\text{True digestibility} = \frac{\text{Dietary AA } (\mu g/g\,\text{DMI}) - (\text{Ileal AA } (\mu g/g\,\text{DMI}) - \text{Endogenous AA } (\mu g/g\,\text{DMI}))}{\text{Dietary AA } (\mu g/g\,\text{DMI})} \quad (2)$$

Calculations for human data.

For 2 of the protein sources (WPI and zein), the collection of digesta related to dietary flow was complete after 9 h (17), and the true ileal AA values were, therefore, calculated based on complete collection of ileal digesta, using Equation 3.

$$\text{True digestibility} = \frac{\text{Dietary AA (mg)} - (\text{Ileal AA (mg)} - \text{Endogenous AA (mg)})}{\text{Dietary AA (mg)}} \quad (3)$$

The pig diets contained both TiO_2 and AIA as markers, and the digestibility results calculated using each marker were compared (data not reported here). For the protein sources bread and bovine collagen, the added celite appeared to mix well within the food matrix, and there was little difference in the AA digestibility results in the pig determined using the 2 markers; thus, for the human study, the digestibility results for these 2 protein sources were calculated based on the determined AIA concentrations (Equations 1 and 2).

For the remaining protein sources, however, the celite was not well distributed throughout the food matrix, and there was poor agreement within the pig data for digestibility determined using AIA and TiO_2. The AIA digestibility values were highly variable, with several values being untenably high or low. For these protein sources in the human, the AIA data were not used. Three of the protein sources (pigeon peas, black beans, and wheat bran) were subjected to a further human digestibility study (human study 2) using TiO_2 as the indigestible marker. For each of these 3 protein sources, total TiO_2 recovery over the 9 h of digesta collection was determined and found to be less than complete and to differ with protein source, although there was a low variation in TiO_2 recovery between participants for the same protein source. Therefore, for pigeon peas, black beans, and wheat bran, digestibility was determined with reference to the marker TiO_2 (study 2), and the determined recovery of titanium (Ti) for each protein source (study 2) was used to calculate AA digestibility values for the first human study corrected to the same recovery (Ti recovery), using Equation 3. The protocols for the first and second human studies were identical with the exception of the marker used.

Statistical analyses

Before the study commenced, a power calculation was carried out using the data from Deglaire et al. (2) for true ileal nitrogen digestibility in the human and pig to determine the required number (n) of human participants and pigs, as well as detect a significant difference between species where means differ by ≥4% and with a SD of 1.97%. Using a power level of 80% and α of 0.05, the minimum n required was 5 human participants and 5 pigs.

The statistical analyses were performed using the statistical software SAS (SAS/STAT version 9.4; SAS Institute). Independent 2-sample t tests were performed to compare the endogenous AA losses between ileal cannulated growing pigs and ileostomate adult humans. The normal distribution and homogeneity of variance for the t tests were evaluated with the use of the ODS Graphics. Amino acids that did not exhibit homogeneity of variance (tryptophan and arginine) were transformed using the natural log before analysis. A t test was also used to compare true ileal lysine digestibility for each diet.

A 2-factor ANOVA model was used to determine the effects of species (human, pig), protein source (black beans, bread, collagen, pigeon peas, wheat bran, whey protein isolate, zein), and species × protein source interaction on the true ileal digestibility of each individual AA, the mean for the indispensable AAs, the mean for the dispensable AAs, and the mean for the total amino acids. For the 2-factor ANOVA, the PROC MIXED routine was used.

The model diagnostics for each analysis were tested using the ODS Graphics procedure and the Repeated statement of SAS. The Repeated statement in PROC MIXED allowed testing for the homogeneity of variance by fitting models with the restricted maximum likelihood method and comparing them using the log-likelihood ratio test. The selected model for all response variables had similar Studentized residuals (i.e., equal variances) across treatments. When the F value of the model was significant ($P \leq 0.05$), the means were compared using least significant difference.

Pearson correlation and simple linear regression analyses were conducted on the human and pig true ileal digestibility values for

each AA using the PROC CORR and PROC REG procedures, respectively.

Simple linear regression equations were derived for the mean true ileal AA digestibilities of the indispensable AAs for each food, determined in the human and pig.

Results

Pig study

The pigs remained healthy throughout the study. The pigs weighed 30.5 ± 0.30 kg at the beginning of the study and 73.3 ± 1.42 kg (mean ± SEM) at the end of the study. Overall, the assay period was 42 d for 6 pigs and 49 d for the remaining 6 pigs, with a total study duration of 65 d.

Human ileostomates

The ileostomates remained healthy throughout the study. At Wageningen UR, 2 participants did not complete the study because of difficulties in ingesting the meals and were replaced by 2 other participants such that the final number of participants was 8. One participant in the Riddet Institute study did not complete the first study. Thus, for WPI and zein, the final number of participants was 8, whereas for pigeon peas, black beans, and wheat bran, $n = 5$ for study 1 combined with $n = 3$ from study 2, giving a final number of participants of 8 over both studies. For bovine collagen and toasted wheat bread, there were 5 participants (from study 1).

For the second human study involving the indigestible marker TiO_2, there was a low degree of variation in Ti recovery between participants for the same protein source. For example, for the protein-free diet, titanium recoveries for the 9-h digesta collection from each of the 3 participants were 44%, 46%, and 45%; for the wheat bran, the Ti recoveries for the 9-h digesta collection were 67%, 65%, and 58%. Other workers have reported relatively short mouth to stoma transit times and low day-to-day variation in transit time (18). There were no statistically significant ($P > 0.05$) differences in AA digestibility for any AA, when comparison was made between study 1 ($n = 5$) and study 2 ($n = 3$), so data were pooled across studies for further analysis.

The gut ileal endogenous AA outputs were significantly greater ($P \leq 0.05$) in the human than the pig for all AAs except the dispensable AA alanine (**Table 2**). When the endogenous outputs were compared in the human between the different human studies, no statistically significant differences were found (data not shown).

The true ileal amino digestibility values determined in the growing pig and adult human are shown graphically in **Figure 1** and the outcome of the ANOVA comparing digestibility for the 7 protein sources in **Table 3**. For most of the AAs, the interaction term (species × protein source) was not significant ($P > 0.05$). There was a significant ($P < 0.001$) effect of protein source for all AAs. The effect of species was generally nonsignificant ($P > 0.05$) except for lysine (conventional analysis), but this effect was not seen for reactive lysine, which provides more accurate values for the lysine content for cooked foods. For tryptophan and serine, the interaction term of the ANOVA comparing digestibility between the human and pig (Table 3) was significant ($P = 0.045$ and 0.047 for tryptophan and serine, respectively), but when the true ileal digestibility means were compared within each protein source separately, there was no significant ($P > 0.05$) effect of species for either of the AAs. For true ileal total lysine digestibility, there was no statistically significant effect of species when protein sources were analyzed separately, except for black beans, for which digestibility values determined in the human were greater (5.2% units) than those determined in the pig. The human ileal lysine digestibility values were often highly variable (CV ranged from 1.7% for WPI to 22% for toasted wheat bread). The pig true ileal lysine digestibilities generally demonstrated lower variability than those for the humans (CV ranged from 1.2% for WPI to 10% for toasted wheat bread).

TABLE 2 Endogenous ileal amino acid losses determined in the adult human and growing pig[1]

Characteristic	Dry matter intake, μg/g		
	Human	Pig	*P* value[2]
Histidine	289 ± 29.1	173 ± 15.7	0.001
Isoleucine	478 ± 47.0	327 ± 26.6	0.007
Leucine	809 ± 70.3	535 ± 43.5	0.002
Lysine	601 ± 59.1	416 ± 42.0	0.016
Methionine	232 ± 36.5	133 ± 12.2	0.004
Phenylalanine	561 ± 44.8	348 ± 28.6	<0.001
Tyrosine	455 ± 46.6	333 ± 25.5	0.021
Threonine	872 ± 76.0	505 ± 37.9	<0.001
Tryptophan	263 ± 30.3	134 ± 11.9	<0.001
Valine	731 ± 85.3	471 ± 38.6	0.006
Alanine	659 ± 70.9	532 ± 50.3	0.15
Arginine	949 ± 159	375 ± 35.1	<0.001
Aspartic acid	1290 ± 98.9	800 ± 61.6	<0.001
Glutamic acid	1310 ± 92.2	885 ± 84.8	0.003
Serine	741 ± 58.7	460 ± 38.8	<0.001

[1] Values are mean ± SEM. For human data, $n = 14$, from studies conducted at Wageningen UR and the Riddet Institute; for pig data, $n = 6$.
[2] *P* value determined using independent 2-sample *t* tests.

The pig/human true ileal AA digestibilities were generally highly correlated (Table 3) with statistically significant ($P \leq 0.05$) correlations for all AAs except threonine, tyrosine, and serine, and for the latter AAs, the correlations tended toward significance ($P = 0.07$ for threonine, $P = 0.13$ for tyrosine and serine).

Overall, when the numerical values for true ileal AA digestibility were compared between the human and pig (Figure 1), for some protein sources (pigeon peas, toasted wheat bread, WPI), mean values for some AAs were greater in the human, whereas mean values for other AAs were greater in the pig. For the protein sources black beans and toasted wheat bread, in particular, several of the mean true ileal AA digestibility values were numerically greater in the human than those in the pig, although overall differences were not statistically significant (mean of essential AAs, $P = 0.923$). For these protein sources, the overall mean true ileal indispensable AA digestibility values were 0.82 and 0.78 in black beans for the human and pig, respectively, whereas for toasted wheat bread, the overall mean true ileal indispensable AA digestibility values were 0.94 and 0.94 for the human and pig, respectively. For the protein sources collagen and zein, almost all of the mean true ileal AA digestibility values were numerically greater in the pig than in the human and to a practically meaningful extent, with overall mean true ileal indispensable AA digestibility values for collagen of 0.75 and 0.81 for the human and pig, respectively, and for zein, the overall mean true ileal indispensable AA digestibility values were 0.60 and 0.67 for the human and pig, respectively. Importantly, when examining the digestibility

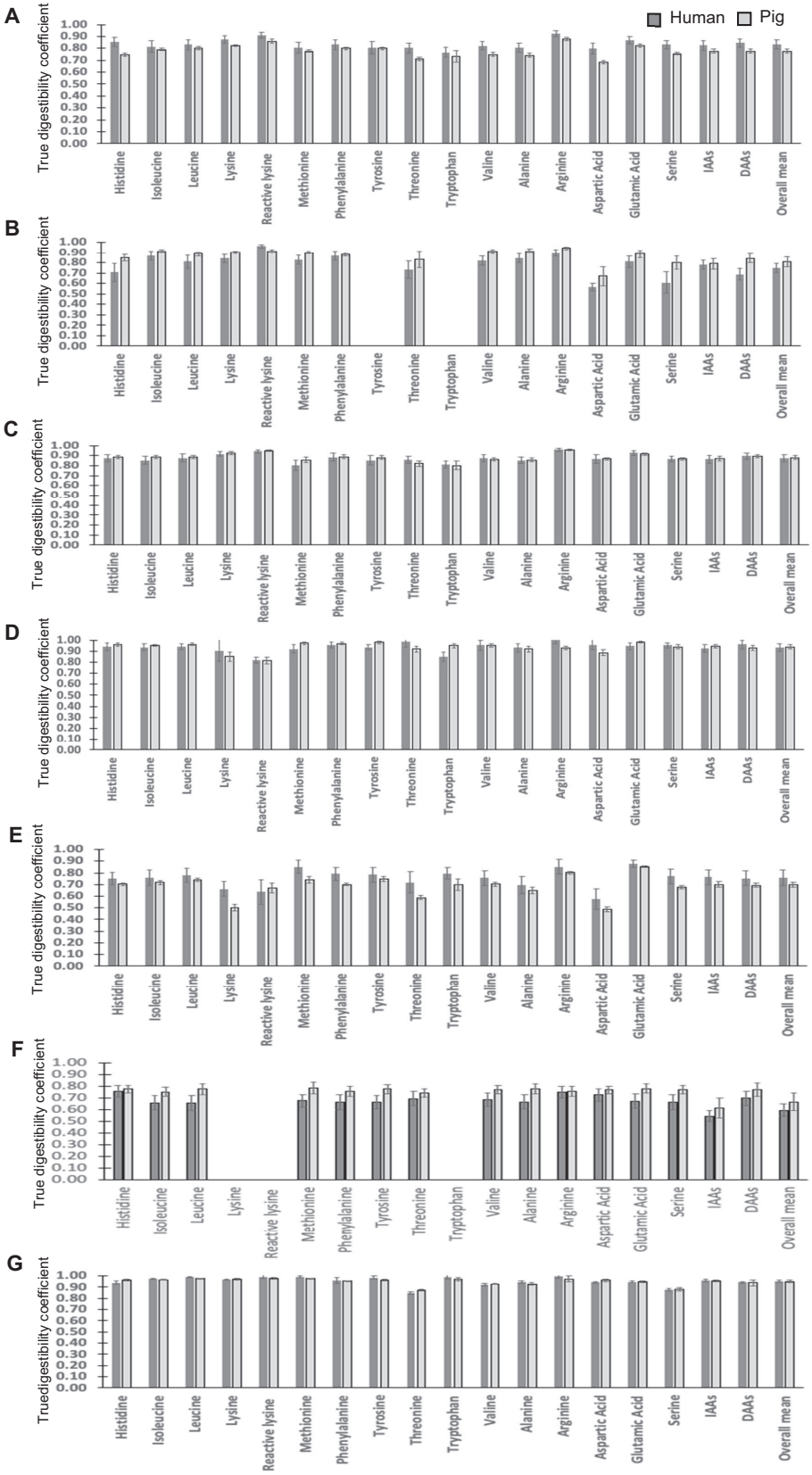

FIGURE 1 True ileal (mean ± SEM) amino acid (AA) digestibility coefficients determined in the adult human and growing pig for black beans (A, $n = 8$ human study, $n = 6$ pig study), collagen (B, $n = 5$ human study, $n = 6$ pig study), pigeon peas (C, $n = 8$ human study, $n = 6$ pig study), toasted wheat bread (D, $n = 5$ human study, $n = 6$ pig study), wheat bran (E, $n = 8$ human study, $n = 6$ pig study), whey protein isolate (F, $n = 8$ human study, $n = 6$ pig study), and zein (G, $n = 8$ human study, $n = 6$ pig study). Overall mean = mean of all AAs (reactive lysine is included when pertinent but lysine is not included in these averages). DAA, dispensable amino acid; IAA, indispensable amino acid.

TABLE 3 Statistical comparison of true ileal amino acid digestibility coefficients determined in the human and pig (black beans, bread, collagen, pigeon peas, wheat bran, whey protein isolate, and zein)[1]

	P value				
Characteristic	Species[2]	Protein source[2]	Interaction[2]	*r*[3]	*P* value
Histidine	0.401	<0.0001	0.120	0.700	0.050
Isoleucine	0.583	<0.0001	0.579	0.905	0.002
Leucine	0.519	<0.0001	0.401	0.848	0.008
Total lysine	0.030	<0.0001	0.118	0.924	0.003
Reactive lysine	0.805	<0.0001	0.222	0.947	0.001
Methionine	0.511	<0.0001	0.254	0.708	0.049
Phenylalanine	0.275	<0.0001	0.744	0.855	0.007
Tyrosine	0.679	<0.0001	0.502	0.584	0.128
Threonine	0.092	<0.0001	0.597	0.675	0.066
Tryptophan	0.360	<0.0001	0.045	0.817	0.047
Valine	0.584	<0.0001	0.463	0.784	0.021
Alanine	0.813	<0.0001	0.175	0.798	0.018
Arginine	0.063	<0.0001	0.280	0.873	0.005
Aspartic acid	0.190	<0.0001	0.111	0.703	0.050
Glutamic acid	0.538	<0.0001	0.173	0.829	0.011
Serine	0.352	<0.0001	0.047	0.589	0.125
M IAAs	0.923	<0.0001	0.423	0.950	<0.001
M DAAs	0.338	<0.0001	0.240	0.706	0.050
Overall mean[4]	0.175	<0.0001	0.488	0.908	0.002

[1] For all pig data, $n = 6$. For human data: black beans, $n = 8$; bread, $n = 5$; collagen, $n = 5$; pigeon peas, $n = 8$; wheat bran, $n = 8$; whey protein isolate, $n = 8$; zein, $n = 8$. M DAAs, mean of dispensable amino acids; M IAAs, mean of indispensable amino acids.
[2] Determined using 2-factor ANOVA model.
[3] Correlation for true ileal amino acid digestibility between pig and human across the seven protein sources.
[4] Overall mean = mean of all amino acids (reactive lysine is included but lysine is not included in these averages).

results for the different protein sources, there was no consistent difference in direction for any of the numerical differences found between species.

The linear regression equations derived (y = human, x = pig), for the mean of the true ileal AA digestibilities of the indispensable AAs expressed as coefficients, were $y = 1.03x - 0.022$; for the mean of the dispensable AAs, $y = 0.889x + 0.090$; and for the overall mean of all AAs, $y = 1.00x - 0.010$. In all cases, the slopes were not statistically significantly different ($P > 0.05$) from unity, and the intercepts did not differ significantly ($P > 0.05$) from zero. The coefficients of determination (R^2) for the respective equations were 0.903, 0.498, and 0.825.

Discussion

Although it would be ideal scientifically, it is impractical to determine true ileal AA digestibility values required for the calculation of DIAAS for all foods with human participants. While it is possible to recruit willing ileostomates to study small numbers of foods, this is not a routine assay that can be frequently undertaken by the ileostomate subject. Moreover, currently, no in vitro AA digestibility method has had the testing and validation required to accurately and confidently determine true ileal AA digestibility values that would be equivalent to values determined in the human. For these reasons, an animal model is required for routine AA digestibility determination, and the growing pig is the recommended first-choice animal model (1, 19).

The adult human and growing pig have similar upper (mouth to ileum) gastrointestinal systems both anatomically and physiologically (9, 20, 21). These similarities support the use of the pig as an animal model for the human when studying digestion between the mouth and the terminal ileum, particularly in terms of protein digestion and absorption. Despite such similarities, however, the growing pig model needs to be validated. To do this, it is possible to determine and compare AA digestibility in ileal cannulated pigs and ileostomized adult humans, but is the ileostomate a suitable model for humans with an intact digestive system? In this respect, adult ileostomates have been used previously in several studies as a model for the "intact" human to provide information on the digestion and absorption of nutrients to the end of the small intestine, for both proteins (8, 22) and fiber (23), and there is considerable evidence that the ileostomy model offers a direct and quantitatively accurate approach to determine nutrient digestibility in the upper gastrointestinal tract (22, 24–31). One difference between ileostomates and humans with an intact digestive tract that has been suggested as being potentially important is an increased colonization of the small intestine by microbes in ileostomates. Englyst and Cummings (26) evaluated polysaccharide digestion in adult ileostomates, including the addition of metronidazole, which inhibits the metabolism of anaerobic flora of the gut, and concluded that there was no significant amount of fermentation in ileostomates. Fuller et al. (32) collected digesta from adult ileostomates both before and after the administration of antibiotics and found no statistically significant differences in the amounts of endogenous ileal amino acids. Sandberg et al. (22) examined dietary fiber breakdown in adult ileostomates and also concluded that there is little if any fermentation. Moreover, the time taken for the "head" of a meal to reach the terminal ileum of the ileostomate has been shown to be the same as the time required for a meal to travel from the mouth to the cecum for "intact" subjects (33). These studies support the adult

ileostomate as being representative of the healthy human with a normal digestive tract.

A means of testing the validity of protein digestibility values obtained using ileostomates is to make a comparison with values obtained using the nasoileal intubation method in participants with a normal intact digestive system. The true ileal AA digestibility of zein and WPI (wide range in digestibilities) from the same batches of material used in the present study was also determined by Calvez et al. (17) using nasoileal intubation in adult humans. The digestibility values for each AA determined in the adult ileostomate (present study) were statistically compared with values determined using nasoileal intubation as reported by Calvez et al. (17). No statistically significant differences were found ($P > 0.05$) for any of the AAs. The overall mean true ileal digestibility of AAs was 0.92 compared with 0.95 for WPI and 0.63 compared with 0.60 for zein [Calvez et al. (17) and data reported in this article]. Both nasoileal intubation and the use of ileostomates to determine true ileal AA digestibility rely on assumptions (6). They are, however, the only 2 models available to directly determine true ileal AA digestibility in the human. In this case, both models gave the same results for the 2 protein sources evaluated.

The biggest complication for studies involving ileostomates is identifying a sufficient number of them who do not have other health concerns that would compromise the results and are willing to participate in the study. In most cases, nowadays, patients who require an ileostomy later have this reversed. This means there is only a small window of time between them being healthy, following the procedure and its cause, and the ileostomy being reversed. Furthermore, the present study was logistically demanding, involving the participants eating the provided breakfast on each study day and then only consuming liquids for the following 9 h.

The AA compositions of the different protein sources included in the present study fell within the range of previously published AA compositions for these foods. For the growing pig, the protein sources from the same batches as used in the present study were also evaluated for true ileal AA digestibility using the same method as used here at Wageningen UR and the University of Illinois (data not shown). The between-site variation in digestibility was low, with a CV for the mean of the indispensable AA digestibilities of 2.7% and an overall mean AA digestibility CV of 2.8% between sites. There was also low variation in the determined AA composition of the protein sources between the sites. This provides confidence in the results obtained in the present study for both the AA composition of the protein sources and true ileal AA digestibility values determined in the pig.

True ileal AA digestibility coefficients have been determined in the adult human and growing pig in previous studies but mainly for highly digestible foods (2, 8). There was no systematic difference between species for true ileal AA digestibilities in these 2 previous controlled comparison studies. Rowan et al. (8) determined human digestibility values for a mixed diet working with human ileostomates and reported that human digestibility values were slightly greater ($P \leq 0.05$) than those obtained in the growing ileostomized pig for threonine, tyrosine, phenylalanine, and methionine. No differences between humans and pigs were observed for any of the other AAs or for total nitrogen. Deglaire et al. (2) used nasoileal intubation in humans and reported that true digestibility values for histidine, lysine, phenylalanine, and tyrosine in the pig for casein and hydolyzed casein were slightly greater than those in the adult human, but no differences for other indispensable AAs were observed. Rapeseed isolate, with a lower nitrogen digestibility, was also evaluated by Deglaire et al. (2), and no statistically significant differences were found for true ileal nitrogen digestibility in the human (0.87) and pig (0.91). For the latter protein source, AA digestibility was not determined because of the low quantity of digesta collected from the human participants. Taken together, the results of these controlled interspecies comparisons show close agreement for true ileal AA digestibility between adult humans and the growing pig. However, the FAO Expert Consultation (1), although acknowledging this agreement between species, concluded that the number of protein sources that had been evaluated previously was too low to give firm evidence of agreement between species.

TABLE 4 Correlation coefficients for true ileal amino acid digestibility values determined in the human and pig for 7 foods from the present study and including 3 historical results[1]

Characteristic	Correlation coefficient (r)	P value
Histidine	0.808	0.003
Isoleucine	0.928	<0.001
Leucine	0.908	<0.001
Lysine	0.937	<0.001
Reactive lysine	0.947	0.001
Methionine	0.708	0.049
Phenylalanine	0.903	<0.001
Tyrosine	0.728	0.011
Threonine	0.820	0.002
Tryptophan	0.854	0.015
Valine	0.857	<0.001
Alanine	0.869	<0.001
Arginine	0.883	0.002
Aspartic acid	0.774	0.005
Glutamic acid	0.865	<0.001
Serine	0.712	0.014
M IAAs	0.966	<0.001
M DAAs	0.791	0.004
Overall mean[2]	0.939	<0.001

[1] Historical results for casein and hydrolyzed casein (2) and a mixed diet (8). M DAAs, mean of dispensable amino acids; M IAAs, mean of indispensable amino acids.
[2] Overall mean = mean of all amino acids (reactive lysine is included but lysine is not included in these averages).

The presently reported results confirm the previously reported observations. In the present study, the protein sources that were evaluated in the human and pig were from the same batch. Factors such as particle size and preparation and cooking were the same for both humans and pigs, to ensure as closely as possible that the human participants and pigs were consuming the same material. It is well established that particle size affects ileal digestibility in the pig (34). Foods that are composed of larger particles, such as black beans and pigeon peas, were lightly mashed in the present study, to ensure adequate mixing with the indigestible marker (celite and/or TiO_2). Marker homogeneity is an essential aspect for accurate estimation of nutrient digestibilities. For some of the foods studied here, a homogeneous distribution of celite was not achieved with mixing, and thus TiO_2 was the preferred indigestible marker. The pig diets included both celite and TiO_2, which allowed an evaluation of their behavior (particularly celite) in the different food matrices. The digestibility and bioavailability of AAs are also affected by heat treatments applied to food compared with the same, untreated food (35, 36), and for this reason, care

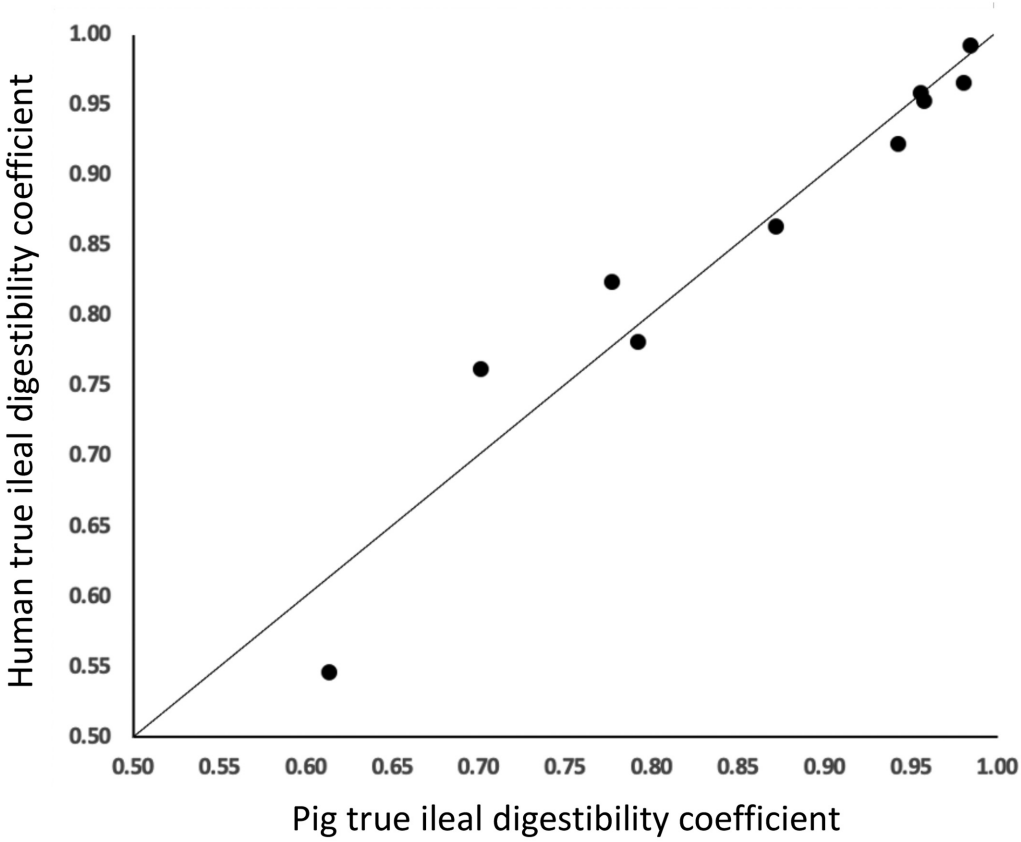

FIGURE 2 Mean true ileal digestibility coefficients for the indispensable amino acids determined in the adult human and growing pig for a range of protein sources (black beans, bread, collagen, pigeon peas, wheat bran, whey protein isolate, and zein from the present study and 3 historical results: casein, hydrolyzed casein, and a mixed diet). The line drawn is y = x.

was taken to prepare the foods in the same manner for each species.

Most previous pig or rat studies that have determined the true ileal AA digestibility of the protein sources that were evaluated in the present study did not prepare the protein sources in the same manner as they would be consumed by humans, making direct comparisons difficult. For example, in many studies, protein sources were evaluated in the raw state but are consumed cooked, or they were tested after being finely ground when they are not normally ground before being consumed by humans. Rutherfurd et al. (14), however, evaluated prepared foods in India, including curries based on pigeon peas, in the rat and obtained similar results to those found in the present study [pigeon pea curry (Sambar) overall mean true ileal digestibility of all AAs was 0.87 compared with 0.88 for pigeon peas in the present study]. Rutherfurd et al. (14) also evaluated raw pigeon peas using the rat. There was a very notable difference in the digestibility of the AAs in the raw and cooked pigeon peas in a curry (overall mean digestibility of 0.58 and 0.87, respectively), highlighting the need to evaluate protein sources in the format that they are consumed.

In the present study, human digesta were found to contain a statistically significantly higher concentration of all gut endogenous AAs except for alanine compared with pig digesta. Values found in the present study for the pig were similar to those reported in previous studies when pigs have received a protein-free diet (37). Endogenous flows were not reported in the 2 previous studies that compared AA digestibilities between the human and pig (2, 8), although Deglaire et al. (2), after collecting digesta via nasoileal intubation, did report that there appeared to be a higher proportion of endogenous protein to total ileal protein in humans compared with the pig, which is consistent with this finding. Deglaire and Moughan (9) reviewed the literature on ileal endogenous nitrogen and AAs and concluded in agreement with the present findings that human ileal digesta contains higher amounts of endogenous protein compared with the growing pig.

When the studies reported here were originally designed, 2 further protein sources were included: sorghum and chickpeas. The consumption of sorghum by the human participants was low (20–30% amount offered) due to a bitter aftertaste that proved difficult to mask. Digestibility values for sorghum were found to be highly variable between participants (e.g., the overall average true ileal AA digestibility ranged from 0.45 to 0.88, and results were spread through this interval). Given the low consumption of sorghum by the human participants, the data for this protein source were not included. For chickpeas, there was poor agreement in digestibility results when data were calculated for the pig using TiO_2 and AIA as markers. It appears that the added celite was not well distributed throughout the food matrix. The AIA digestibility values were highly variable, with several values being untenably high or low. Thus, the chickpea data were not reported or included in the analyses.

When the true ileal amino acid digestibility results were compared between the human and pig, of all the indispensable amino acids, there was only a significant species effect for total lysine (but not reactive lysine), and when the data for total lysine were compared within protein source, the only food for which the true ileal lysine digestibility results were significantly different between species was black beans (5% unit

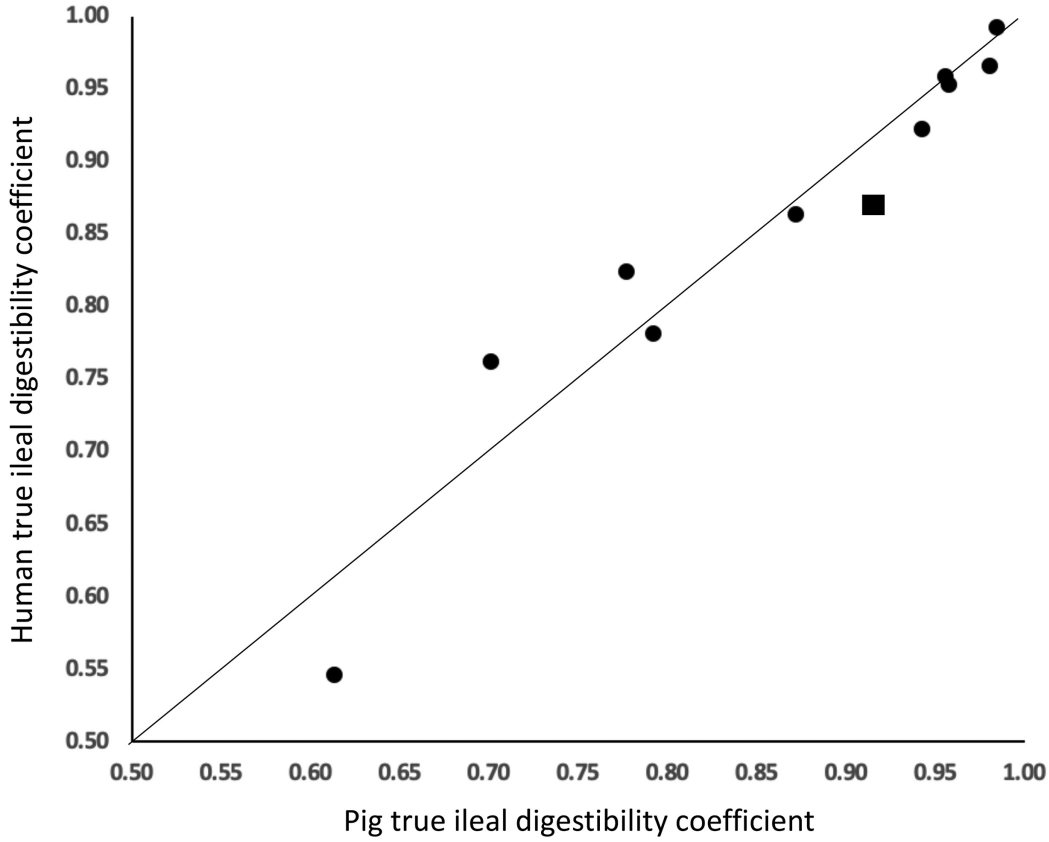

FIGURE 3 Mean true ileal digestibility coefficients for the indispensable amino acids determined in the adult human and growing pig for a range of protein sources (black beans, bread, collagen, pigeon peas, wheat bran, whey protein isolate, and zein from the present study and 3 historical results: casein, hydrolyzed casein, and a mixed diet). True ileal digestibility of nitrogen from rapeseed is also included [black square, historical result; Deglaire et al. (2)]. The line drawn is y = x.

difference). There were no statistically significant differences for true ileal AA digestibility for any other protein source. To put this in context, out of >100 (16 AAs × 7 proteins) digestibility comparisons, only 1 such interspecies difference was statistically significantly different. The results of this study, providing a wider range in digestibility compared with earlier work, confirms the suitability of the growing pig as an animal model for the adult human to determine true ileal AA digestibility. Importantly, there was no systematic difference between the results for true ileal AA digestibility in the human and pig. Generally, true ileal AA digestibility was highly correlated between pigs and humans. The linear regression equations that were derived (y = human, x = pig), for the mean of the true ileal AA digestibilities of the indispensable AAs expressed as coefficients (y = 1.03x – 0.022; for the mean of the dispensable AAs, y = 0.889 x + 0.090; and for the overall mean of all AAs, y = 1.00x – 0.010), are very close to y = x.

The digestibility data from previously published studies involving 3 further protein sources (a mixed human diet, casein, and hydrolyzed casein) (2, 8) were added to the present data set and the data reanalyzed with the 10 protein sources to evaluate the correlation for digestibility between the human and pig (**Table 4**). The correlation coefficients between the human and pig were high and statistically significant for all AAs. The mean true ileal digestibility coefficients for the indispensable AAs are shown in **Figure 2**. It can be seen from Figure 2 that for AA digestibilities in the range of 80–100% (most foodstuffs), there is evidence for very close interspecies agreement. At lower digestibilities, however, there was greater divergence between species, although the mean digestibility results were not different statistically, and there was no directional bias. It was noted that variation between individuals was relatively high for the ileostomates receiving these more poorly digested foods (wheat bran, zein), and it may be that inherent limitations of the ileostomate model, such as potential bacterial colonization, intestinal resection, and morphologic gut changes (38), are potentially more important when the digestive process is more challenged with low digestible foods.

The 2011 FAO Expert Consultation anticipated potentially small interspecies differences and envisaged a linear regression equation being developed relating true ileal AA digestibility in the adult human to that in the growing pig. In practice, the equation would be applied to pig data, to give "predicted" human AA digestibility. In this case, linear regression equations were derived for the data presented in Figure 2 for overall mean true ileal AA digestibility for the indispensable and dispensable AAs. The regression equations between the human (y) and pig (x) (coefficients of digestibility) were as follows: for the mean of the true ileal AA digestibilities of the indispensable AAs, y = 1.001x – 0.008 (R^2 = 0.933); for the mean of the dispensable AAs, y = 0.915x – 0.070 (R^2 = 0.626); and for the overall mean of all AAs, y = 0.991x – 0.001 (R^2 = 0.882). These equations were, as discussed above for the more restricted analysis, very close to y = x, confirming that it is not necessary to apply regression equations to data obtained in the pig to estimate human true ileal AA digestibility values. For the indispensable AAs, the equations show that for a pig digestibility coefficient of 0.80, the predicted human

digestibility value is 0.793, and for a pig value of 0.90, the predicted human value is 0.893. For completeness, a further human/pig digestibility relation incorporating previously published data is given in **Figure 3**, where the true ileal digestibility of total nitrogen for rapeseed isolate (2), given as a proxy for true ileal indispensable AA digestibility, is provided. The regression equation for the data presented in Figure 3 is (y = human, x = pig) $y = 0.995x + 0.0004$, $R^2 = 0.922$.

Given that the pig and predicted human digestibility values are very close in magnitude, this means that values determined in the cannulated growing pig can be used directly. This means that the large data set of true ileal AA digestibility of foods and feedstuffs that already exists is translatable to a human data set, without requiring mathematical adjustments. It is important, however, to ensure that the previous pig studies were conducted in a similar manner to that described by Hodgkinson et al. (10) and that the foods/ingredients/protein sources were fed to the pigs in the same form (processing, cooking, particle size) as they are consumed by humans. Data from foods that were fed to pigs in a raw state but that are consumed in a cooked state by humans, or that were ground before feeding to the pigs when they are not typically ground when consumed by humans, cannot be considered valid for inclusion in such a database. In addition, the true ileal AA digestibility coefficient calculations should have been made with corrections for endogenous AA flows done on an individual pig basis as opposed to using the overall mean endogenous AA flows.

It is important to note that as well as being used to calculate DIAAS, the contents of true ileal digestible AAs can be used to evaluate combinations of food sources based on their full digestible AA profile. True ileal digestible AAs are additive when foods are mixed (39).

There is currently a paucity of data on true ileal AA digestibility for foods commonly consumed in developing countries and for many food groups, such as fruit and vegetables. There is a major international scientific collaboration (Proteos Project) under way that aims to populate a large data set of the AA digestibility of foods as consumed by humans using the cannulated pig model. This will allow for an orderly implementation of the DIAAS measure. The present results confirm the validity of the cannulated growing pig model.

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