

Digestible indispensable amino acid scores of nine cooked cereal grains

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Abstract

True ileal digestibility (TID) values of amino acid (AA) obtained using growing rats are often used for the characterisation of protein quality in different foods and acquisition of digestible indispensable amino acid scores (DIAAS) in adult humans. Here, we conducted an experiment to determine the TID values of AA obtained from nine cooked cereal grains (brown rice, polished rice, buckwheat, oats, proso millet, foxtail millet, tartary buckwheat, adlay and whole wheat) fed to growing Sprague–Dawley male rats. All rats were fed a standard basal diet for 7 d and then received each diet for 7 d. Ileal contents were collected from the terminal 20 cm of ileum. Among the TID values obtained, whole wheat had the highest values ($P < 0.05$), and polished rice, proso millet and tartary buckwheat had relatively low values. The TID indispensable AA concentrations in whole wheat were greater than those of brown rice or polished rice ($P < 0.05$), and polished rice was the lowest total TID concentrations among the other cereal grains. The DIAAS was 68 for buckwheat, 47 for tartary buckwheat, 43 for oats, 42 for brown rice, 37 for polished rice, 20 for whole wheat, 13 for adlay, 10 for foxtail millet and 7 for proso millet. In this study, the TID values of the nine cooked cereal grains commonly consumed in China were used for the creation of a DIAAS database and thus gained public health outcomes.

Key words: Cooked cereal grains: Digestible indispensable amino acid scores: True ileal digestibility: Protein quality: Growing rats

Accurately estimating the dietary protein and amino acid (AA) digestibility of food products is necessary⁽¹⁾. The protein digestibility-corrected amino acid score (PDCAAS) has been adopted by the Joint FAO/WHO Expert Consultation since 1991 and has since been used for the evaluation of protein quality in food products⁽²⁾. However, this method has several limitations^(3,4). The main difference between the newly recommended digestible indispensable AA score (DIAAS) and PDCAAS is that the true ileal AA digestibility for the dietary indispensable AA is used in DIAAS rather than a single faecal crude protein (CP) digestibility value. AA are absorbed from the small intestine only and are metabolised extensively by the microbiota of the hindgut. Terminal ileal digestibility is more accurate than faecal digestibility in estimating AA bioavailability^(5–8). Moreover, PDCAAS underestimates the comparatively high nutritional values of some proteins by truncation and overestimates the quality of proteins containing anti-nutritional factors and limiting AA^(9–14). In contrast, DIAAS is not truncated for a single-source protein and is preferred to PDCAAS for the evaluation of protein quality by the FAO⁽¹⁵⁾. Digestibility should be based on the true ileal digestibility (TID) of each AA,

which is preferably determined in humans, but if this is not possible, TID can be determined in growing pigs or rats⁽¹⁶⁾.

Cereal grains are often the main component of the human diet and provide a large proportion of the dietary protein for humans, especially in developing countries⁽¹⁷⁾. Thus, accurately assessing the protein nutritional value of cereal grains is essential⁽¹¹⁾. Cereal grains and grain by-products are usually cooked before human consumption. Directly determining ileal AA digestibility in humans is difficult and expensive; thus, the Expert Consultation (FAO, 2013) recommended the use of pigs, which are the best models for adult humans; alternatively, growing rat can also be used^(15,18,19). In this study, we aimed to determine the apparent ileal digestibility (AID), TID values of AA and DIAAS values in nine cooked cereal grains fed to growing rats.

Methods

Materials

Brown rice, polished white rice, oats, tartary buckwheat, buckwheat, foxtail millet, proso millet, adlay and wheat

Abbreviations: AA, amino acid; AID, apparent ileal digestibility; CP, crude protein; DIAAS, digestible indispensable amino acid score; PDCAAS, protein digestibility-corrected amino acid score; TID, true ileal digestibility.

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were used. Adlay was purchased from the Guizhou Province, while oats and foxtail millet were procured from Inner Mongolia. The other cereal grains were obtained from Northwest A&F University. Wheat was baked into wheat bread according to the national standard (LST 3204-1993). The other cereal grains were soaked for 30 min with 25°C deionised water. The cereals were then cooked using a commercially available cooker as described by the manufacturer. The respective proportions of brown rice, polished white rice, oats, tartary buckwheat, buckwheat, foxtail millet, proso millet or adlay to water were 1:1.6, 1:1.6, 1:2.3, 1:20, 1:20, 1:1.8, 1:1.9 or 1:1.4 (w/v), respectively. All the cooked materials were freeze dried after cooking, and all the materials were ground through a size-60 mesh before inclusion into the diets.

Animals and diets

The animal experiments used 150 male Sprague–Dawley rats that were approximately 240 g in weight and were purchased from the Beijing Vital River Laboratory Animal Center. Rats were caged individually and were maintained under controlled temperature ($22 \pm 2^\circ\text{C}$), humidity and airflow condition, with a 12-h on–off light cycle as described by Rutherford *et al.*⁽³⁾. Adequate measures were taken to minimise the pain or discomfort of the rats, and we used the smallest possible number of animals. The study was reviewed and approved by the Institutional Animal Ethics Committee at Jiangnan University (JN. No. 20170930k1201105 [36]). All animals were maintained according to local regulations and guidelines.

A total of eleven semisynthetic wheat starch-based diets (Table 1) were formulated to contain 100 g/kg CP, which was the sole protein source. To meet the requirements for growing rats, we added vitamins and minerals. A total of 3 g/kg of titanium dioxide was included in each diet as an indigestible marker. Purified sucrose, soyabean oil and cellulose were mixed in a ratio of 10:5:3 (180 g/kg DM). To maintain a dietary CP concentration of 100 g/kg for low-protein foods with CP concentration of <150 g/kg DM, the test ingredient was diluted with cellulose and soyabean oil (1:0.6); for foods with CP concentration of <100 g/kg DM, the test diet consists of the test ingredient, vitamin/mineral mixture and titanium dioxide⁽²⁰⁾. The ingredient compositions of the basal and test diets are shown in Table 1. A protein-free-based diet was prepared for rats to determine the amount of endogenous loss of AA in the ileal content⁽²¹⁾. A basal diet containing 100 g/kg protein was also formulated using casein as the sole source of protein⁽²²⁾.

Experimental design

The rats (n 150) were randomly divided into 10 groups (n 15/group) as follows: brown rice group, polished rice group, buckwheat group, oats group, proso millet group, foxtail millet group, tartary buckwheat group, adlay group, whole wheat group and protein-free-based diet group. All rats were initially

fed a standard basal diet for 7 d. After 1 week of acclimatisation period, the experimental groups received each diet in Table 1 for 7 d. Each rat received its respective diet in nine hourly meals (08.30–16.30 hours) daily. The diet was freely available for 10 min at each meal time. Water was also freely available. On the 14th day of the study, each rat was killed 5 h after the first meal through asphyxiation with CO_2 gas^(3,23). Ileal contents were immediately collected from the terminal 20 cm of ileum. Given that the ileal content of each rat is insufficient for the AA detection through HPLC⁽²²⁾, three ileum contents were mixed into one sample in each group (n 5). All ileal content samples were freeze-dried and frozen (-20°C) while awaiting chemical analysis.

Chemical analysis

CP content was determined by rapid N cube (NY/T 2007–2011) using a N-to-protein conversion factor of 6.25. The AA contents were determined in triplicate 5-mg samples following hydrolysis in 500 μl of constant-boiling HCl (6 mol/l) for 24 h at $110 \pm 1^\circ\text{C}$ in a hydrolysis tube⁽²⁴⁾. The liberated AA were derived with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, and α -aminobutyric acid was used as the internal standard. The derivatives were separated on a Waters E2695 HPLC system equipped with a C18 column (150 mm \times 4.6 mm, 5.0 μm ; Agilent) and quantified using Waters 2475 fluorescence detector at 395 nm emission and 250 nm excitation. To determine cysteine and methionine, we used performic acid oxidation at 0°C for 16 h, followed by neutralisation with HBr; then, we applied hydrolysis as described above. The concentration of titanium in the diets and ileal samples was determined through the method described by Short *et al.*⁽²⁵⁾. The samples were ashed, then digested in 60% (v/v) sulfuric acid and finally added to 30% hydrogen peroxide. Absorbance at 410 nm was measured. Tryptophan (Trp) was determined using the method described by Rutherford & Gilani⁽²⁴⁾. Free AA molecular weights were used for the calculation of the weight of each AA.

Data analysis

AA and CP contents in the terminal ileal digesta and the TID of AA were calculated by using the equation given by Rutherford *et al.*⁽²⁶⁾. In addition, the endogenous ileal AA flows were determined for rats fed the protein-free diet⁽²⁷⁾.

Apparent and true ileal AA digestibility was calculated using the following equations (units are g/kg DM intake)^(6,28):

$$\text{AID}_{\text{AA}} = 1 - \left(\frac{\text{AA}_{\text{digesta}}}{\text{AA}_{\text{diet}}} \times \left(\frac{\text{Ti}_{\text{diet}}}{\text{Ti}_{\text{digesta}}} \right) \right) \times 100,$$

where AID_{AA} is the AID of AA (%), $\text{AA}_{\text{digesta}}$ is the concentration of AA in the ileal digesta DM, AA_{diet} is the concentration of AA in the diet DM, Ti_{diet} is the concentration of Ti in the diet DM and $\text{Ti}_{\text{digesta}}$ is the concentration of Ti in the ileal digesta DM.

$$\text{TID}_{\text{AA}} = \text{AID} + \left(\frac{\text{IAA}_{\text{end}}}{\text{AA}_{\text{diet}}} \times 100 \right),$$

where IAA_{end} is the ileal endogenous AA losses.

$$\text{DIAA reference ratio} = \frac{\text{mg of the digestible dietary indispensable AA in 1 g of the test protein}}{\text{mg of the dietary indispensable AA in 1 g of the reference protein}}$$

where the reference protein indispensable AA profile was the AA requirement pattern for the 0.5–3 years old child⁽¹⁵⁾.

DIAAS was calculated using the following equation^(15,20):

$$\text{DIAAS (\%)} = 100 \times \frac{\text{lowest value of the digestible indispensable AA reference ratio}}{\text{AA reference ratio}}$$

Statistical analysis

Calculation of sample size was performed using the 'resource equation' method, as described by Charan & Kantharia⁽²⁹⁾, with a power of 80% and significance of 5%. Results were expressed as mean values with their standard errors. The Shapiro–Wilk comparison normality test was used to assess the distribution of all variables. Comparisons for normally distributed data between the two groups were conducted using two-tailed *t* test and one-way ANOVA followed by Tukey's significance test for multiple comparisons. Mann–Whitney *U* and Kruskal–Wallis tests were used for non-parametric analysis when data were non-normally distributed. A *P* value of <0.05 was considered significant. All statistical calculations were performed on SPSS 21.0 data processing software (SPSS Inc.).

Results

Crude protein and amino acid compositions of nine cooked cereal grains

A total of eighteen AA were detected in nine cooked cereal grains. The total AA concentrations of the nine cooked cereal grains on an as-fed basis ranged from 8.3% (polished rice) to 18.5% (adlay; Table 2). The CP contents of the cooked cereal grains ranged from 9.15% (polished rice) to 19.28% (adlay). The CP contents of buckwheat, oats, proso millet, foxtail millet, adlay and whole wheat were higher (*P*<0.05) than those of brown rice, polished rice and tartary buckwheat. The cooked cereal grains had indispensable AA contents, ranging from 30.5 (brown rice) to 66.3 g/kg DM (adlay). The AA compositions in the diets based on the nine cooked cereal grains are shown in Table 3.

Mean apparent ileal digestibility of amino acids in nine cooked cereal grains

The mean AID of indispensable AA in whole wheat was greater than that in any of the other cooked cereal grains (Table 4). The AID values of most AA in whole wheat were nonsignificantly different from those in adlay, except that the AID of leucine (Leu) in whole wheat was lower than that in adlay (*P*<0.05), whereas the AID of lysine (Lys) in whole wheat was higher than that in adlay (*P*<0.05). The mean AID of the indispensable AA and AID of Lys and Trp in proso-millet were the lowest among the values obtained for all cooked cereal grains. The mean AID of the indispensable AA and AID of all indispensable AA in

proso millet were significantly lower (*P*<0.05) than that in foxtail millet. The mean AID of all AA in adlay were all greater than those in all the other cooked cereal grains, except whole wheat. Meanwhile, the mean AID of all AA in polished rice and proso millet were lowest among the values obtained for all cooked cereal grains (*P*<0.05).

Mean true ileal digestibility of amino acids in nine cooked cereal grains

The mean TID of indispensable AA in whole wheat and adlay were greater than those for other cooked cereals (*P*<0.05; Table 5). Furthermore, no difference was observed in the mean TID of indispensable AA between adlay and whole wheat. No difference was observed between the indispensable AA TID values of buckwheat and foxtail millet, although Leu and Trp TID values were greater (*P*<0.05) in foxtail millet than in buckwheat. The mean TID of the indispensable AA in polished rice, proso millet and tartary buckwheat were lower (*P*<0.05) than those of the other cooked cereal grains.

Mean true ileal digestibility concentrations for amino acids in nine cooked cereal grains

The total TID concentrations of indispensable AA in buckwheat were significantly lower than those for adlay, foxtail millet, proso millet and oats and significantly greater than that for brown rice, tartary buckwheat and polished rice (*P*<0.05; Table 6). Adlay had the highest TID concentrations of valine, isoleucine, Leu and tyrosine among the cooked cereal grains (*P*<0.05), and buckwheat and brown rice had the highest TID concentrations of Lys and Trp, respectively (*P*<0.05). Polished rice had the lowest total TID concentration of indispensable AA (*P*<0.05).

Digestible indispensable amino acid score for nine cooked cereal grains

The following DIAAS values were obtained: 42, brown rice; 37, polished rice; 68, buckwheat; 43, oats; 7, proso millet; 10, foxtail millet; 47, tartary buckwheat; 13, adlay and 20, whole wheat (Table 7).

Discussion

The nine cereal grains tested in this study are commonly produced in different provinces in China. Buckwheat and tartary buckwheat belonging to Polygonaceae family grow mainly in Russia, China and India⁽³⁰⁾. Proso millet (*Panicum miliaceum* L.) is consumed as a staple food among the majority of people who live in arid and semi-arid tropics of the world, such as Asia, Africa and some parts of Europe⁽³¹⁾. Foxtail millet (*Setaria italica*) is one of the most important food crops of the semi-arid tropics in



Table 1. Ingredient composition of the experimental diet (g/kg DM)

Composition	Brown rice	Polished rice	Buckwheat	Oats	Proso millet	Foxtail millet	Tartary buckwheat	Adlay	Whole wheat	Protein-free diet	Basal diet
Wheat starch	–	–	88.0	104.0	171.0	–	–	233.0	157.0	752.0	637.0
Soyabean oil	–	–	50.0	50.0	50.0	96.9	–	50.0	50.0	50.0	50.0
Purified cellulose	–	–	30.0	30.0	30.0	58.1	–	30.0	30.0	30.0	30.0
Purified sucrose	–	–	100.0	100.0	100.0	–	–	100.0	100.0	100.0	100.0
Vitamin trace element mix*	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5
Mineral mix†	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Choline chloride	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Cooked brown rice	932.0	–	–	–	–	–	–	–	–	–	–
Cooked polished white rice	–	932.0	–	–	–	–	–	–	–	–	–
Cooked buckwheat	–	–	664.0	–	–	–	–	–	–	–	–
Cooked oats	–	–	–	648.0	–	–	–	–	–	–	–
Cooked proso millet	–	–	–	–	581.0	–	–	–	–	–	–
Cooked foxtail millet	–	–	–	–	–	777.0	–	–	–	–	–
Cooked tartary buckwheat	–	–	–	–	–	–	932.0	–	–	–	–
Cooked adlay	–	–	–	–	–	–	–	519.0	–	–	–
Wheat bread	–	–	–	–	–	–	–	–	595.0	–	–
Enzyme-hydrolysed casein	–	–	–	–	–	–	–	–	–	–	–
Lactic casein	–	–	–	–	–	–	–	–	–	–	115.0
Titanium dioxide	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total (g)	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Digestible indispensable amino acid scores

* The vitamins and trace elements are as follows: 250 mg retinol; 1.8 mg cholecalciferol; 1185 mg α -tocopherol; 1808 mg thiamine; 312 mg riboflavin; 2338 mg niacin; 2058 mg pantothenic acid; 312 mg pyridoxine; 1.8 mg cyanocobalamin; 125 mg phyloquinone; 93.9 mg folic acid; 4.56 g Mn; 10.29 g Fe; 904 mg Cu; 3273 mg Zn; 41 mg iodine; 7.5 mg Se; 39 mg Co.
 † The mineral mix of the diet includes 25 g CaPO₄, 5.3 g CaCO₃, 3.6 g NaCl, 12.5 g KCl and 3.6 g MgSO₄.

Table 2. Determined crude protein (CP) and amino acid (AA) compositions of cooked brown rice, polished rice, buckwheat, oats, proso millet, foxtail millet, tartary buckwheat, adlay and whole wheat (g/kg DM)*
(Mean values with their standard errors)

	Brown rice		Polished rice		Buckwheat		Oats		Proso millet		Foxtail millet		Tartary buckwheat		Adlay		Whole wheat		P	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM		
CP	96.04 ^e	1.78	91.54 ^e	1.08	150.53 ^c	2.1	154.33 ^c	2.85	172.14 ^b	4.26	128.66 ^d	1.43	94.67 ^e	2.23	192.81 ^a	4.35	168.23 ^b	2.94	0.05	
Indispensable AA																				
His	2.08 ^e	0.03	2.21 ^{d,e}	0.02	3.55 ^a	0.09	2.98 ^c	0.07	2.95 ^c	0.10	2.28 ^d	0.07	2.30 ^d	0.03	3.24 ^b	0.10	3.60 ^a	0.06	0.05	
Thr	3.07 ^f	0.04	3.05 ^f	0.07	5.69 ^a	0.14	4.52 ^c	0.06	4.11 ^d	0.05	4.20 ^d	0.10	3.65 ^e	0.12	4.78 ^b	0.11	4.62 ^{b,c}	0.12	0.05	
Val	4.96 ^e	0.07	4.94 ^e	0.12	7.41 ^b	0.26	7.15 ^{b,c}	0.13	7.04 ^c	0.10	5.57 ^d	0.12	50.00 ^e	0.05	9.54 ^a	0.14	6.87 ^c	0.09	0.05	
Met	1.66 ^f	0.06	2.02 ^d	0.05	1.94 ^{d,e}	0.03	1.84 ^e	0.02	3.22 ^b	0.04	3.50 ^a	0.11	1.03 ^g	0.12	2.20 ^c	0.07	1.64 ^f	0.04	0.05	
Lys	2.79 ^e	0.04	2.67 ^e	0.04	8.56 ^a	0.17	5.03 ^c	0.09	0.78 ^h	0.01	1.18 ^g	0.04	5.27 ^b	0.10	2.27 ^f	0.07	4.44 ^d	0.13	0.05	
Ile	3.28 ^g	0.09	3.49 ^g	0.13	5.58 ^c	0.18	5.26 ^d	0.11	5.99 ^b	0.11	4.66 ^e	0.15	4.01 ^f	0.11	7.10 ^a	0.09	5.59 ^c	0.10	0.05	
Leu	7.07 ^f	0.15	7.02 ^f	0.07	10.06 ^e	0.12	10.74 ^d	0.24	20.21 ^b	0.24	15.06 ^c	0.23	6.90 ^f	0.16	26.10 ^a	0.28	10.79 ^d	0.16	0.05	
Phe	4.39 ^f	0.12	4.68 ^{e,f}	0.10	7.19 ^d	0.12	7.65 ^c	0.16	9.05 ^b	0.10	6.92 ^d	0.15	4.73 ^e	0.05	9.83 ^a	0.23	7.55 ^c	0.15	0.05	
Trp	1.16 ^d	0.02	0.76 ^f	0.02	1.83 ^a	0.05	1.15 ^d	0.03	1.28 ^c	0.03	1.54 ^b	0.03	1.52 ^b	0.04	1.27 ^c	0.03	1.05 ^e	0.03	0.05	
Dispensable AA																				
Asp	7.86 ^f	0.21	7.42 ^f	0.12	13.88 ^a	0.39	11.22 ^c	0.19	8.17 ^e	0.20	7.88 ^{e,f}	0.14	9.00 ^e	0.10	11.89 ^b	0.30	8.12 ^e	0.15	0.05	
Ser	4.50 ^g	0.06	4.43 ^g	0.11	7.47 ^c	0.27	7.01 ^d	0.09	9.68 ^a	0.21	5.35 ^e	0.17	4.90 ^f	0.10	8.51 ^b	0.23	7.62 ^c	0.14	0.05	
Glu	15.87 ^g	0.23	15.31 ^g	0.21	26.50 ^e	0.83	31.05 ^d	0.46	33.27 ^c	0.40	23.58 ^f	0.44	14.96 ^g	0.17	43.93 ^b	0.49	46.79 ^a	0.90	0.05	
Gly	4.16 ^d	0.10	3.81 ^e	0.09	8.44 ^a	0.27	6.77 ^b	0.12	2.63 ^g	0.06	3.04 ^f	0.04	5.26 ^c	0.09	4.27 ^d	0.10	6.75 ^b	0.12	0.05	
Arg	7.51 ^d	0.33	7.30 ^d	0.12	15.21 ^a	0.18	9.64 ^b	0.19	4.73 ^e	0.07	4.21 ^f	0.12	8.69 ^c	0.14	8.62 ^c	0.19	8.46 ^c	0.19	0.05	
Ala	4.92 ^f	0.07	4.61 ^f	0.12	6.09 ^{d,e}	0.07	6.32 ^d	0.11	15.51 ^b	0.39	10.06 ^c	0.10	4.11 ^g	0.11	17.62 ^a	0.24	5.73 ^e	0.06	0.05	
Pro	4.34 ^g	0.06	4.13 ^g	0.05	6.17 ^f	0.09	8.06 ^e	0.15	11.25 ^c	0.14	9.24 ^d	0.15	3.61 ^h	0.08	15.21 ^b	0.22	18.05 ^a	0.29	0.05	
Cys	1.05 ^{d,e}	0.03	1.14 ^d	0.04	2.72 ^b	0.06	2.91 ^b	0.10	0.91 ^{e,f}	0.01	3.92 ^a	0.10	0.83 ^f	0.01	0.89 ^{e,f}	0.01	2.18 ^c	0.22	0.05	
Tyr	3.88 ^f	0.12	4.31 ^e	0.13	4.37 ^e	0.05	5.20 ^c	0.08	5.88 ^b	0.09	3.82 ^f	0.11	2.77 ^g	0.04	7.62 ^a	0.21	4.89 ^d	0.16	0.05	
Total indispensable AA	30.47 ^f	0.63	30.83 ^f	0.48	51.82 ^c	1.17	46.33 ^d	0.91	54.64 ^b	0.76	44.9 ^d	0.99	34.40 ^e	0.78	66.33 ^a	0.98	46.14 ^d	0.87	0.05	
Total dispensable AA	54.10 ^f	1.21	52.47 ^f	0.98	90.86 ^{c,d}	2.20	88.19 ^d	1.49	92.04 ^c	1.55	71.09 ^e	1.39	54.14 ^f	0.85	118.53 ^a	2.00	108.6 ^b	2.24	0.05	
Total AA	84.57 ^f	1.85	83.30 ^f	1.47	142.68 ^c	3.37	134.52 ^d	2.40	146.68 ^c	2.31	115.99 ^e	2.38	88.54 ^f	1.62	184.86 ^a	2.98	154.74 ^b	3.11	0.05	

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a,b,c,d,e,f,g,h Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
* Based on triplicate determinations. CP was based on a N-to-protein conversion factor of 6.25.

Table 3. Determined amino acid (AA) compositions of brown rice, polished rice, buckwheat, oats, proso millet, foxtail millet, tartary buckwheat, adlay and whole wheat-based diets (g/kg DM)* (Mean values with their standard errors)

	Brown rice		Polished rice		Buckwheat		Oats		Proso millet		Foxtail millet		Tartary buckwheat		Adlay		Whole wheat		P
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Indispensable AA																			
His	1.89 ^e	0.03	1.60 ^f	0.03	2.14 ^b	0.04	2.01 ^{c,d}	0.03	2.08 ^{b,c}	0.05	1.89 ^e	0.06	2.04 ^{b,c}	0.03	1.92 ^{d,e}	0.06	2.28 ^a	0.05	0.05
Thr	2.78 ^e	0.03	2.46 ^f	0.03	3.46 ^a	0.06	3.13 ^c	0.04	2.99 ^d	0.07	3.33 ^b	0.05	3.23 ^{b,c}	0.06	2.75 ^e	0.03	2.95 ^d	0.09	0.05
Val	4.61 ^b	0.07	4.18 ^c	0.05	4.61 ^b	0.15	5.17 ^a	0.05	5.26 ^a	0.20	4.15 ^c	0.09	4.54 ^b	0.07	5.40 ^a	0.14	4.51 ^b	0.06	0.05
Met	0.96 ^d	0.03	1.62 ^c	0.03	1.17 ^d	0.02	0.64 ^e	0.07	2.15 ^b	0.35	3.05 ^a	0.05	1.02 ^d	0.01	2.07 ^b	0.04	1.10 ^d	0.02	0.05
Lys	2.31 ^d	0.03	1.97 ^e	0.03	4.89 ^a	0.18	3.07 ^c	0.08	0.36 ⁱ	0.00	0.70 ^h	0.01	4.40 ^b	0.08	0.92 ^g	0.02	1.32 ^f	0.01	0.05
Ile	3.09 ^e	0.06	2.85 ^f	0.04	3.39 ^d	0.12	3.71 ^c	0.07	4.49 ^a	0.09	4.08 ^b	0.05	3.58 ^c	0.04	4.01 ^b	0.11	3.66 ^c	0.10	0.05
Leu	6.83 ^e	0.10	6.24 ^f	0.11	6.48 ^{e,f}	0.11	7.94 ^c	0.14	15.37 ^a	0.46	13.20 ^b	0.21	6.24 ^f	0.11	15.44 ^a	0.32	7.39 ^d	0.10	0.05
Phe	4.54 ^{d,e}	0.06	3.93 ^f	0.05	4.59 ^d	0.15	5.53 ^b	0.08	6.76 ^a	0.19	5.72 ^b	0.09	4.31 ^e	0.10	5.65 ^b	0.15	4.98 ^c	0.09	0.05
Trp	1.94 ^a	0.07	0.75 ^f	0.02	1.11 ^d	0.03	1.36 ^c	0.03	0.94 ^e	0.01	1.78 ^b	0.03	1.37 ^c	0.03	0.95 ^e	0.02	0.99 ^e	0.02	0.05
Dispensable AA																			
Asp	7.07 ^c	0.09	6.52 ^d	0.16	8.87 ^a	0.29	7.92 ^a	0.09	6.05 ^e	0.13	6.46 ^d	0.08	8.08 ^a	0.10	6.68 ^d	0.17	5.23 ^f	0.08	0.05
Ser	4.14 ^d	0.06	3.85 ^e	0.04	4.76 ^c	0.12	5.16 ^b	0.06	7.25 ^a	0.18	4.29 ^d	0.07	4.36 ^d	0.05	4.82 ^c	0.16	4.94 ^{b,c}	0.11	0.05
Glu	14.52 ^f	0.25	14.00 ^f	0.22	17.04 ^e	0.60	22.36 ^c	0.32	24.90 ^b	0.79	19.51 ^d	0.21	13.52 ^f	0.19	24.59 ^b	0.51	30.75 ^a	0.36	0.05
Gly	3.79 ^e	0.05	3.30 ^f	0.08	5.43 ^a	0.14	5.16 ^b	0.11	1.86 ^h	0.04	2.39 ^g	0.07	4.69 ^c	0.05	2.44 ^g	0.06	4.43 ^d	0.08	0.05
Arg	7.27 ^c	0.19	6.04 ^d	0.08	9.81 ^a	0.14	7.16 ^c	0.14	1.48 ^h	0.03	2.39 ^g	0.07	7.63 ^b	0.13	4.22 ^f	0.13	5.29 ^e	0.11	0.05
Ala	4.71 ^d	0.05	4.38 ^e	0.04	3.88 ^f	0.14	4.72 ^d	0.06	12.19 ^a	0.20	8.41 ^c	0.18	3.83 ^f	0.10	10.31 ^b	0.17	3.71 ^f	0.10	0.05
Pro	4.12 ^e	0.06	3.51 ^f	0.05	3.68 ^f	0.05	5.83 ^d	0.08	8.63 ^b	0.25	7.90 ^c	0.15	3.20 ^g	0.03	8.91 ^b	0.13	11.54 ^a	0.18	0.05
Cys	0.62 ^c	0.01	0.62 ^c	0.02	1.12 ^b	0.02	1.42 ^a	0.25	0.60 ^c	0.01	0.69 ^c	0.01	0.61 ^c	0.02	0.59 ^c	0.01	1.06 ^b	0.02	0.05
Tyr	3.57 ^{b,c}	0.06	3.37 ^d	0.05	2.58 ^e	0.04	3.62 ^b	0.11	4.36 ^a	0.12	3.39 ^{c,d}	0.09	2.43 ^e	0.09	4.36 ^a	0.09	3.24 ^d	0.04	0.05
Total indispensable AA	28.95 ^e	0.08	25.61 ^f	0.39	31.84 ^{c,d}	0.66	32.58 ^c	0.60	40.41 ^a	1.40	37.91 ^b	0.65	30.72 ^d	0.52	39.11 ^{a,b}	0.58	29.17 ^e	0.35	0.05
Total dispensable AA	49.80 ^e	0.09	45.60 ^f	0.34	57.18 ^d	1.32	63.36 ^c	1.04	67.32 ^b	1.75	56.35 ^d	0.89	48.36 ^e	0.77	66.92 ^b	1.41	70.19 ^a	1.08	0.05
Total AA	78.75 ^e	0.01	71.21 ^f	0.73	89.02 ^d	1.98	95.93 ^{b,c}	1.63	107.74 ^a	3.14	94.25 ^c	1.53	79.08 ^e	1.29	106.03 ^a	1.98	99.36 ^b	1.43	0.05

Digestible indispensable amino acid scores

^{a,b,c,d,e,f,g,h} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* Based on triplicate determinations. Crude protein was based on a N-to-protein conversion factor of 6.25.

Table 4. Mean apparent ileal digestibility of amino acid (AA) in brown rice, polished rice, buckwheat, oats, proso millet, foxtail millet, tartary buckwheat, adlay and whole wheat (%) (Mean values with their standard errors)

	Brown rice		Polished rice		Buckwheat		Oats		Proso millet		Foxtail millet		Tartary buckwheat		Adlay		Whole wheat			P
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	SEM	
Indispensable AA																				
His	80.93 ^{a,b}	2.76	68.11 ^d	4.82	78.88 ^{b,c}	3.37	78.03 ^{b,c}	4.44	74.34 ^c	4.18	81.69 ^{a,b}	4.03	67.18 ^d	3.95	81.98 ^{a,b}	3.67	85.46 ^a	3.65	2.11	0.05
Thr	64.51 ^{b,c}	3.32	42.67 ^f	3.31	67.01 ^{a,b}	2.09	60.81 ^c	3.08	49.63 ^e	2.74	65.10 ^{b,c}	2.45	55.11 ^d	2.73	69.54 ^{a,b}	3.36	70.70 ^a	5.59	3.19	0.05
Val	77.15 ^c	1.43	72.36 ^d	1.84	78.48 ^{b,c}	1.70	79.88 ^{b,c}	1.72	73.21 ^d	2.30	81.02 ^b	1.88	70.74 ^d	2.14	87.43 ^a	1.58	85.59 ^a	1.68	1.92	0.05
Met	56.60 ^d	0.90	55.44 ^d	4.82	84.91 ^{a,b}	1.17	82.12 ^b	1.28	65.96 ^c	1.42	86.90 ^{a,b}	4.57	64.77 ^c	7.62	89.19 ^a	3.91	88.69 ^a	2.16	4.69	0.05
Lys	77.63 ^{a,b}	3.61	73.93 ^{a,b,c}	3.81	79.70 ^a	1.63	71.39 ^{a,b,c}	2.65	17.25 ^f	2.37	36.18 ^e	11.65	65.55 ^c	1.94	50.97 ^d	3.58	68.56 ^{b,c}	2.61	7.07	0.05
Ile	73.57 ^d	3.89	68.79 ^e	2.71	79.52 ^c	2.47	83.71 ^b	2.11	72.15 ^{d,e}	1.84	79.98 ^c	1.95	71.06 ^{d,e}	2.67	87.75 ^a	2.19	87.86 ^a	2.24	2.41	0.05
Leu	75.97 ^f	1.59	68.16 ^h	1.32	81.11 ^d	1.00	86.10 ^c	1.03	78.83 ^e	1.38	89.68 ^b	1.18	71.84 ^g	1.50	93.84 ^a	1.04	89.08 ^b	2.22	2.90	0.05
Phe	77.42 ^c	2.14	74.57 ^d	1.89	84.87 ^b	1.75	85.37 ^b	1.55	76.27 ^{c,d}	1.23	86.33 ^b	2.62	76.47 ^{c,d}	2.06	91.77 ^a	1.14	90.97 ^a	1.28	2.20	0.05
Trp	83.05 ^{b,c}	1.43	71.99 ^{e,f}	0.91	79.79 ^{c,d}	1.21	74.54 ^e	1.31	70.39 ^f	3.13	86.87 ^a	1.69	78.37 ^d	2.33	83.93 ^{a,b}	2.90	84.22 ^{a,b}	4.03	1.95	0.05
Mean	74.09 ^d	1.41	66.22 ^{e,f}	2.43	79.36 ^{b,c}	1.76	78.00 ^c	1.80	64.23 ^f	2.03	77.08 ^{c,d}	3.12	69.01 ^e	2.78	81.82 ^{a,b}	2.24	83.46 ^a	2.44	2.30	0.05
Dispensable AA																				
Asp	75.98 ^b	1.69	64.03 ^d	2.63	80.30 ^a	1.33	76.89 ^b	1.21	59.49 ^e	1.47	75.97 ^b	2.34	65.92 ^d	1.35	77.65 ^b	1.27	70.71 ^c	1.64	2.40	0.05
Ser	70.59 ^{c,c}	2.24	54.48 ^e	3.79	68.76 ^c	1.25	70.07 ^c	2.05	63.01 ^d	1.32	75.38 ^b	2.07	62.27 ^d	2.22	80.38 ^a	2.41	81.24 ^a	1.87	2.92	0.05
Glu	74.39 ^f	1.17	63.88 ^h	1.42	86.50 ^d	0.82	89.54 ^c	0.67	66.33 ^g	1.09	84.62 ^e	0.96	75.09 ^h	0.74	92.06 ^b	0.81	94.12 ^a	0.47	3.72	0.05
Gly	64.44 ^b	1.45	41.35 ^d	2.73	67.46 ^{a,b}	2.09	65.49 ^b	1.56	9.86 ^f	4.59	31.28 ^e	3.32	52.10 ^c	1.97	41.57 ^d	3.55	69.74 ^a	1.97	6.72	0.05
Arg	87.54 ^{a,b}	1.26	80.20 ^{d,e}	1.67	87.74 ^a	1.47	83.46 ^{b,c,d,e}	1.10	58.75 ^f	5.63	79.37 ^e	2.44	82.58 ^{c,d,e}	1.65	86.56 ^{a,b,c}	3.30	84.07 ^{a,b,c,d}	1.69	2.97	0.05
Ala	77.25 ^b	0.90	67.82 ^d	2.80	72.28 ^c	1.62	77.35 ^b	1.74	74.17 ^c	1.37	85.99 ^a	1.29	66.82 ^d	1.99	86.93 ^a	0.92	79.49 ^b	2.39	2.36	0.05
Pro	70.95 ^c	2.22	59.30 ^e	3.60	66.83 ^d	2.49	80.07 ^b	1.52	69.82 ^{c,d}	1.85	85.17 ^a	1.66	51.45 ^f	3.32	82.88 ^{a,b}	0.86	80.90 ^b	1.07	3.82	0.05
Cys	55.16 ^{d,e}	1.51	48.40 ^f	1.49	75.24 ^b	1.07	87.33 ^a	0.93	52.07 ^{e,f}	3.07	76.50 ^b	4.05	59.14 ^d	4.00	70.18 ^c	5.30	78.00 ^b	2.86	4.52	0.05
Tyr	71.47 ^d	2.49	67.74 ^{d,e}	2.45	80.28 ^{b,c}	3.12	83.50 ^{a,b}	2.22	77.25 ^c	2.45	83.52 ^{a,b}	2.92	64.08 ^e	3.46	83.77 ^{a,b}	1.82	85.07 ^a	2.69	2.61	0.05
Mean	71.97 ^d	0.36	60.80 ^f	1.93	76.16 ^{b,c}	1.52	79.30 ^a	1.21	58.97 ^f	1.98	75.31 ^c	2.03	64.39 ^e	1.90	78.00 ^{a,b}	1.83	80.37 ^a	1.58	2.74	0.05
Total mean	73.03 ^d	0.75	63.51 ^f	2.18	77.76 ^{b,c}	1.57	78.65 ^{b,c}	1.49	61.60 ^f	1.94	76.20 ^c	2.57	66.70 ^e	2.32	79.91 ^{a,b}	1.98	81.92 ^a	2.01	2.50	0.05

^{a,b,c,d,e,f} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

Table 5. Mean true ileal digestibility (TID) of amino acid (AA) in brown rice, polished rice, buckwheat, oats, proso millet, foxtail millet, tartary buckwheat, adlay and whole wheat (%)* (Mean values with their standard errors)

	Brown rice		Polished rice		Buckwheat		Oats		Proso millet		Foxtail millet		Tartary buckwheat		Adlay		Whole wheat			P
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	SEM	
Indispensable AA																				
His	93.12 ^a	2.76	82.46 ^{d,e}	4.82	89.66 ^{a,b}	3.37	89.48 ^{a,b}	4.44	85.42 ^{c,d}	4.18	93.88 ^a	4.03	78.46 ^e	3.95	94.00 ^a	3.67	95.56 ^a	3.65	1.96	0.05
Thr	88.09 ^b	3.32	69.24 ^e	3.31	85.95 ^{b,c}	2.09	81.76 ^c	3.08	71.56 ^e	2.74	84.76 ^{b,c}	2.45	75.41 ^d	2.73	93.39 ^a	3.36	92.92 ^a	5.59	2.94	0.05
Val	85.91 ^c	1.43	82.02 ^c	1.84	87.25 ^a	1.70	87.69 ^a	1.72	80.89 ^b	2.30	90.75 ^a	1.88	79.65 ^b	2.14	94.91 ^a	1.58	94.55 ^a	1.68	1.87	0.05
Met	62.56 ^c	0.90	58.69 ^c	4.82	89.39 ^a	1.17	90.32 ^a	1.28	68.41 ^b	1.42	88.63 ^a	4.57	69.93 ^b	7.62	91.74 ^a	3.91	93.49 ^a	2.16	4.68	0.05
Lys	93.41 ^{a,b}	3.61	92.48 ^{a,b}	3.81	87.15 ^{b,c}	1.63	83.25 ^c	2.65	95.97 ^a	5.37	88.27 ^{b,c}	7.65	73.84 ^d	1.94	90.40 ^{a,b,c}	3.58	96.18 ^a	2.61	2.36	0.05
Ile	82.87 ^e	3.89	78.87 ^f	2.71	88.00 ^{c,d}	2.47	91.45 ^{b,c}	2.11	78.54 ^f	1.84	87.03 ^d	1.95	79.07 ^f	2.67	94.91 ^{a,b}	2.19	95.70 ^a	2.24	2.27	0.05
Leu	83.49 ^d	1.59	76.39 ^d	1.32	89.03 ^c	1.00	92.57 ^b	1.03	82.17 ^d	1.38	93.56 ^b	1.18	80.07 ^e	1.50	97.16 ^a	1.04	96.03 ^a	2.22	2.51	0.05
Phe	83.63 ^c	2.14	81.40 ^d	1.89	90.72 ^b	1.75	90.22 ^b	1.55	80.23 ^{c,d}	1.23	91.01 ^b	2.62	82.69 ^{c,d}	2.06	96.51 ^a	1.14	96.36 ^a	1.28	2.09	0.05
Trp	86.89 ^b	1.43	81.86 ^{c,d}	0.91	86.50 ^b	1.21	80.01 ^{d,e}	1.31	78.31 ^e	3.13	91.05 ^a	1.69	83.82 ^{b,c}	2.33	91.74 ^a	2.90	91.75 ^a	4.03	1.70	0.05
Mean	84.44 ^c	1.41	78.16 ^d	2.43	88.18 ^b	1.76	87.42 ^{b,c}	1.80	80.17 ^d	2.03	89.88 ^b	3.12	78.11 ^d	2.78	93.86 ^a	2.24	94.73 ^a	2.44	2.11	0.05
Dispensable AA																				
Asp	89.87 ^{a,b}	1.69	79.03 ^c	2.63	91.36 ^{a,b}	1.33	89.28 ^b	1.21	75.72 ^d	1.47	91.17 ^{a,b}	2.34	78.06 ^{c,d}	1.35	92.33 ^a	1.27	89.64 ^{a,b}	1.64	2.21	0.05
Ser	88.84 ^c	2.24	74.04 ^f	3.79	84.63 ^d	1.25	84.69 ^d	2.05	73.42 ^f	1.32	93.00 ^b	2.07	79.58 ^e	2.22	96.06 ^{a,b}	2.41	96.52 ^a	1.87	2.92	0.05
Glu	82.29 ^e	1.17	72.07 ^f	1.42	93.23 ^c	0.82	94.67 ^b	0.67	70.89 ^f	1.09	90.50 ^d	0.96	83.59 ^e	0.74	96.73 ^a	0.81	97.85 ^a	0.47	3.41	0.05
Gly	95.13 ^a	1.45	76.62 ^c	2.73	88.90 ^b	2.09	88.07 ^d	1.56	72.34 ^d	4.59	80.05 ^c	3.32	76.90 ^c	1.97	89.31 ^b	3.55	96.00 ^a	1.97	2.86	0.05
Arg	92.97 ^{a,b}	1.26	86.74 ^d	1.67	91.76 ^{b,c}	1.47	88.97 ^{b,c,d}	1.10	85.40 ^d	5.63	91.27 ^{b,c}	2.44	87.76 ^{b,c,d}	1.65	95.91 ^a	3.30	91.53 ^{b,c}	1.69	1.11	0.05
Ala	85.49 ^b	0.90	76.68 ^d	2.80	82.29 ^c	1.62	85.56 ^b	1.74	77.35 ^d	1.37	90.60 ^a	1.29	76.94 ^d	1.99	90.69 ^a	0.92	89.94 ^a	2.39	1.97	0.05
Pro	87.52 ^{b,c}	2.22	78.71 ^d	3.60	85.35 ^c	2.49	91.77 ^a	1.52	77.73 ^d	1.85	93.80 ^a	1.66	72.74 ^e	3.32	90.54 ^{a,b}	0.86	86.81 ^c	1.07	2.38	0.05
Cys	68.84 ^{d,e}	1.51	62.10 ^f	1.49	82.82 ^c	1.07	93.33 ^a	0.93	66.25 ^{e,f}	3.07	88.84 ^b	4.05	73.13 ^d	4.00	84.62 ^{b,c}	5.30	86.04 ^{b,c}	2.86	3.69	0.05
Tyr	78.62 ^c	2.49	75.31 ^c	2.45	90.16 ^a	3.12	90.55 ^a	2.22	83.11 ^b	2.45	91.06 ^a	2.92	74.60 ^c	3.46	89.63 ^a	1.82	92.95 ^a	2.69	2.43	0.05
Mean	85.51 ^c	0.36	75.70 ^d	1.93	87.84 ^{b,c}	1.52	89.65 ^{a,b}	1.21	75.80 ^d	1.98	90.03 ^{a,b}	2.03	78.14 ^d	1.90	91.76 ^a	1.83	91.92 ^a	1.58	2.26	0.05
Total mean	84.97 ^c	0.75	76.93 ^d	2.18	88.01 ^b	1.57	88.54 ^b	1.49	77.98 ^d	1.94	89.96 ^b	2.57	78.12 ^d	2.32	92.81 ^a	1.98	93.32 ^a	2.01	2.16	0.05

Digestible indispensable amino acid scores

^{a,b,c,d,e,f} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

* TID values were calculated by correcting the values for apparent ileal digestibility for the basal endogenous losses. Values used for the basal endogenous losses were follows (g/kg of DM intake): Asp, 1.09; Ser, 0.83; Glu, 1.25; Gly, 1.24; His, 0.26; Arg, 0.42; Thr, 0.73; Ala, 0.43; Pro, 0.76; Cys, 0.09; Tyr, 0.28; Val, 0.44; Met, 0.07; Lys, 0.40; Ile, 0.32; Leu, 0.55; Phe, 0.30; Trp, 0.08.

Table 6. Mean true ileal digestibility concentrations (g/kg DM) for amino acid (AA) in brown rice, polished rice, buckwheat, oats, proso millet, foxtail millet, tartary buckwheat, adlay and whole wheat (Mean values with their standard errors)

	Brown rice		Polished rice		Buckwheat		Oats		Proso millet		Foxtail millet		Tartary buckwheat		Adlay		Whole wheat			P
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	SEM	
Indispensable AA																				
His	1.76 ^c	0.05	1.32 ^e	0.08	1.92 ^b	0.07	1.80 ^{b,c}	0.09	1.78 ^c	0.09	1.77 ^c	0.08	1.60 ^d	0.08	1.80 ^{b,c}	0.07	2.18 ^a	0.08	0.08	0.05
Thr	2.45 ^c	0.09	1.71 ^e	0.08	2.97 ^a	0.07	2.56 ^c	0.10	2.14 ^d	0.08	2.82 ^b	0.08	2.43 ^c	0.09	2.56 ^c	0.09	2.74 ^b	0.16	0.13	0.05
Val	3.96 ^d	0.07	3.43 ^g	0.08	4.02 ^d	0.08	4.54 ^b	0.09	4.26 ^c	0.12	3.77 ^e	0.08	3.61 ^f	0.10	5.12 ^a	0.09	4.26 ^c	0.08	0.17	0.05
Met	0.55 ^f	0.01	0.95 ^d	0.08	1.05 ^d	0.01	0.58 ^f	0.01	1.47 ^c	0.03	2.70 ^a	0.14	0.71 ^e	0.08	1.90 ^b	0.08	1.03 ^d	0.02	0.23	0.05
Lys	2.16 ^d	0.08	1.82 ^e	0.07	4.26 ^a	0.08	2.56 ^c	0.08	0.44 ^j	0.02	0.62 ^h	0.08	3.25 ^b	0.09	0.84 ^g	0.03	1.27 ^f	0.03	0.43	0.05
Ile	2.56 ^f	0.12	2.25 ^g	0.08	2.98 ^d	0.08	3.40 ^c	0.08	3.53 ^{b,c}	0.08	3.55 ^b	0.08	2.83 ^e	0.10	3.80 ^a	0.09	3.51 ^{b,c}	0.08	0.18	0.05
Leu	5.70 ^f	0.11	4.77 ^h	0.08	5.77 ^f	0.07	7.35 ^d	0.08	12.63 ^b	0.21	12.35 ^c	0.16	5.00 ^g	0.09	15.01 ^a	0.16	7.10 ^e	0.16	1.29	0.05
Phe	3.61 ^f	0.09	3.20 ^g	0.07	4.16 ^e	0.08	4.99 ^c	0.09	5.43 ^a	0.08	5.21 ^b	0.15	3.56 ^f	0.09	5.45 ^a	0.06	4.80 ^d	0.06	0.29	0.05
Trp	1.69 ^a	0.03	0.62 ^h	0.01	0.96 ^e	0.01	1.09 ^d	0.02	0.74 ^g	0.03	1.62 ^b	0.03	1.14 ^c	0.03	0.87 ^f	0.03	0.91 ^f	0.04	0.12	0.05
Total	24.44 ^f	0.40	20.06 ^g	0.55	28.10 ^e	0.55	28.86 ^d	0.53	32.41 ^c	0.53	34.42 ^b	0.74	24.15 ^f	0.68	37.36 ^a	0.59	27.78 ^e	0.62	1.81	0.05
Dispensable AA																				
Asp	6.35 ^c	0.12	5.17 ^f	0.17	8.11 ^a	0.12	7.07 ^b	0.10	4.58 ^g	0.09	5.89 ^e	0.15	6.31 ^{c,d}	0.11	6.17 ^d	0.08	4.65 ^g	0.09	0.38	0.05
Ser	3.68 ^e	0.09	2.86 ^g	0.15	4.03 ^d	0.06	4.37 ^c	0.11	5.32 ^a	0.10	3.99 ^d	0.09	3.47 ^f	0.10	4.63 ^b	0.12	4.77 ^b	0.09	0.25	0.05
Glu	11.95 ^f	0.17	10.09 ^h	0.20	15.89 ^e	0.14	21.17 ^c	0.15	17.81 ^d	0.27	17.66 ^d	0.19	11.30 ^g	0.10	23.79 ^b	0.20	30.09 ^a	0.15	2.17	0.05
Gly	3.61 ^b	0.05	2.53 ^e	0.09	4.83 ^a	0.11	4.54 ^b	0.08	1.35 ^h	0.09	1.91 ^g	0.08	3.61 ^d	0.09	2.18 ^f	0.09	4.25 ^c	0.09	0.42	0.05
Arg	6.76 ^b	0.09	5.24 ^d	0.10	9.00 ^a	0.14	6.37 ^c	0.08	1.26 ^h	0.08	3.03 ^g	0.08	6.69 ^b	0.13	4.05 ^f	0.14	4.84 ^e	0.09	0.76	0.05
Ala	4.02 ^c	0.04	3.36 ^d	0.12	3.19 ^e	0.06	4.04 ^c	0.08	9.43 ^a	0.17	7.62 ^b	0.11	2.95 ^f	0.08	9.35 ^a	0.09	3.34 ^d	0.09	0.91	0.05
Pro	3.60 ^f	0.09	2.77 ^h	0.13	3.14 ^g	0.09	5.35 ^e	0.09	6.71 ^d	0.16	7.41 ^c	0.13	2.33 ^j	0.11	8.07 ^b	0.08	10.02 ^a	0.12	0.90	0.05
Cys	0.43 ^{e,f}	0.01	0.39 ^g	0.01	0.93 ^b	0.01	1.32 ^a	0.01	0.40 ^{f,g}	0.02	0.61 ^c	0.03	0.45 ^e	0.02	0.50 ^d	0.03	0.91 ^b	0.03	0.11	0.05
Tyr	2.80 ^e	0.09	2.54 ^f	0.08	2.33 ^g	0.08	3.28 ^c	0.08	3.63 ^b	0.11	3.08 ^d	0.10	1.81 ^h	0.08	3.91 ^a	0.08	3.01 ^d	0.09	0.22	0.05
Total	43.20 ^e	0.20	34.93 ^g	0.57	51.45 ^d	0.75	57.52 ^c	0.65	50.49 ^d	0.83	51.20 ^d	0.74	38.92 ^f	0.69	62.64 ^b	0.72	65.88 ^a	0.71	3.46	0.05
Total AA	67.70 ^f	0.58	54.99 ^h	1.12	79.55 ^e	1.25	86.38 ^c	1.17	82.90 ^d	1.31	85.62 ^c	1.48	63.07 ^g	1.36	100.00 ^a	1.31	93.66 ^b	1.32	3.52	0.05

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^{a,b,c,d,e,f,g,h} Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).



Table 7. Digestible indispensable amino acid scores (DIAAS) for brown rice, polished rice, buckwheat, oats, proso millet, foxtail millet, tartary buckwheat, adlay and whole wheat*

	Brown rice	Polished rice	Buckwheat	Oats	Proso millet	Foxtail millet	Tartary buckwheat	Adlay	Whole wheat
DIAA reference ratio									
His	0.99	0.77	0.89	0.86	0.79	0.84	0.88	0.80	0.97
Thr	0.89	0.64	0.89	0.79	0.61	0.86	0.86	0.73	0.79
Val	1.04	0.93	0.87	1.01	0.88	0.83	0.92	1.05	0.88
Lys	0.42	0.37	0.70	0.43	0.07	0.10	0.63	0.13	0.20
Ile	0.90	0.82	0.87	1.02	0.98	1.05	0.97	1.05	0.98
Leu	0.97	0.84	0.82	1.07	1.70	1.78	0.83	2.01	0.96
Trp	2.23	0.84	1.05	1.23	0.77	1.82	1.48	0.91	0.95
Sulfur AA	0.43	0.58	0.68	0.68	0.62	1.17	0.47	0.78	0.64
Aromatic AA	1.39	1.28	1.17	1.52	1.55	1.51	1.13	1.59	1.34
DIAAS† (%)	42 (Lys)	37 (Lys)	68 (SAA)	43 (Lys)	7 (Lys)	10 (Lys)	47 (SAA)	13 (Lys)	20 (Lys)

DIAA, digestible indispensable amino acid; AA, amino acid.

* DIAAS were calculated for the 0.5–3 years old child.

† Indispensable AA reference patterns are expressed as mg AA/kg protein: His, 16; Ile, 30; Leu, 61; Lys, 48; sulfur AA, 23; aromatic AA, 41; Thr, 25; Trp, 66; Val, 40⁽²⁾.

Asia and Africa⁽³²⁾. Adlay (*Coix lacryma-jobi* L.) is mainly cultivated in China and Japan⁽³³⁾. Many recent studies indicated that the consumption of these cereal grains are beneficial because they reduce the risk of acquiring chronic diseases^(30,32,34–36).

The protein and AA contents of protein sources should be determined and the TID of each indispensable AA in the test protein should be used to allow calculation of accurate DIAAS values⁽¹⁶⁾. Grain proteins play many important roles in human health; thus, assessing their quality after processing is important. A few decades ago, the FAO established a method for protein nutritional value assessment. AA digestibility determination at the terminal ileum is more accurate than the traditional faecal method⁽³⁷⁾. Although ileal digestibility may not be a perfect measure to determine net AA absorption, it is considerably closer than the AA digestibility determined over the total digestive tract⁽³⁸⁾. TID values are usually very accurate unless a protein has been overheated, which may result in reduced digestibility of Lys⁽¹⁵⁾. The variations in the AID values may be a result of the differences among grain varieties and growing conditions of the grains⁽³⁹⁾. Therefore, protein evaluation can be improved by calculating the TID values of AA and removing the influences of basal endogenous losses of AA on determined digestibility values⁽⁶⁾.

In the 2011 Protein Quality Expert Consultation, DIAAS was reported to provide more accurate protein quality scores than the PDCAAS⁽¹⁵⁾. However, nearly all available DIAAS data were obtained from pig models, and those derived from humans remains insufficient⁽¹¹⁾. In this study, the DIAAS values obtained from polished rice, oats and whole wheat were lower than those reported by Cervantes-Pahm *et al.*⁽²⁸⁾, Mathai *et al.*⁽⁴⁰⁾ and Abelilla *et al.*⁽⁴¹⁾. According to the DIAAS cut-off value introduced by an FAO Expert Consultation report and the study performed by Cervantes-Pahm *et al.*^(15,28), only dehulled oats are good protein sources for human consumption because its DIAAS is 77. However, the DIAAS was 68 for buckwheat, 47 for tartary buckwheat and 43 for oats in this study. It is possible that buckwheat and tartary buckwheat are better protein sources than oats. However, further work is needed to compare the digestibility in the rat-based assay to that in human-based studies with the use of the same foods when consumed by humans.

In conclusion, diets based on proso millet and foxtail millet require more AA supplementation than those based on buckwheat, tartary buckwheat, oats and brown rice for them to meet the balanced AA based on DIAAS values in this study. DIAAS value obtained from cereal grains can provide comprehensive nutritional information and a scientific basis for the evaluation of the nutritional values of proteins contained in different cereals. Given the DIAAS values obtained from cereal grains, the rational combination of various cereal grains had increased protein quality in human diets and is useful as a scientific basis for formulating balanced diets.

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The authors declare that there are no conflicts of interest.

References

- Moughan PJ (2012) Dietary protein for human health (Preface). *Br J Nutr* **108**, S1–S2.
- Food and Agriculture Organization of the United Nations (1991) *Protein Quality Evaluation*. Report of the Joint FAO/WHO Expert Consultation, Bethesda, Md., USA, 4–8 December 1989. FAO Food and Nutrition Paper 51. Rome: FAO.
- Rutherfurd SM, Fanning AC, Miller BJ, *et al.* (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.
- Schaafsma G (2012) Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. *Br J Nutr* **108**, S333–S336.
- Moughan PJ & Stevens BR (2012) Digestion and absorption of protein. In *Biochemical, Physiological and Molecular Aspects of Human Nutrition*, pp. 162–178 [MH Stipanuk and MA Caudill, editors]. St Louis, MO: Elsevier.
- Stein HH, Seve B, Fuller MF, *et al.* (2007) Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. *J Anim Sci* **85**, 172–180.
- Moughan PJ (2003) Amino acid availability: aspects of chemical analysis and bioassay methodology. *Nutr Res Rev* **16**, 127–141.
- Rowan AM, Moughan PJ, Wilson MN, *et al.* (1994) Comparison of the ileal and fecal digestibility of dietary amino-acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. *Br J Nutr* **71**, 29–42.
- Schaafsma G (2000) The protein digestibility-corrected amino acid score. *J Nutr* **130**, 1865S–1867S.
- Schaafsma G (2005) The protein digestibility-corrected amino acid score (PDCAAS) – a concept for describing protein quality in foods and food ingredients: a critical review. *JAOAC Int* **88**, 988–994.
- Lee WTK, Weisell R, Albert J, *et al.* (2016) Research approaches and methods for evaluating the protein quality of human foods proposed by an FAO expert working group in 2014. *J Nutr* **146**, 929–932.
- Boye J, Wijesinha-Bettoni R & Burlingame B (2012) Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *Br J Nutr* **108**, S183–S211.
- Millward DJ, Layman DK, Tome D, *et al.* (2008) Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. *Am J Clin Nutr* **87**, 1576S–1581S.
- Gilani GS, Xiao CW & Cockell KA (2012) Impact of anti-nutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *Br J Nutr* **108**, S315–S332.
- Food and Agriculture Organization of the United Nations (2013) Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. FAO Food and Nutrition Paper 92. http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ff_c17f06.pdf
- Wolfe RR, Rutherfurd SM, Kim IY, *et al.* (2016) Protein quality as determined by the digestible indispensable amino acid score: evaluation of factors underlying the calculation. *Nutr Rev* **74**, 584–599.
- Bwibo NO & Neumann CG (2003) The need for animal source foods by Kenyan children. *J Nutr* **133**, 3936S–3940S.
- Deglaire A & Moughan PJ (2012) Animal models for determining amino acid digestibility in humans – a review. *Br J Nutr* **108**, S273–S281.
- Butts CA, Monro JA & Moughan PJ (2012) *In vitro* determination of dietary protein and amino acid digestibility for humans. *Br J Nutr* **108**, S282–S287.
- Food and Agriculture Organization of the United Nations (2014) *Research Approaches and Methods for Evaluating the Protein Quality of Human Foods: Report of a FAO Expert Working Group*. Rome: FAO.
- Moughan PJ & Rutherfurd SM (2012) Gut luminal endogenous protein: implications for the determination of ileal amino acid digestibility in humans. *Br J Nutr* **108**, S258–S263.
- Rutherfurd SM & Moughan PJ (2003) The rat as a model animal for the growing pig in determining ileal amino acid digestibility in soya and milk proteins. *J Anim Physiol Anim Nutr* **87**, 292–300.
- Rutherfurd SM & Moughan PJ (1998) The digestible amino acid composition of several milk proteins: application of a new bioassay. *J Dairy Sci* **81**, 909–917.
- Rutherfurd SM & Gilani GS (2009) Amino acid analysis. *Curr Protoc Protein Sci* **58**, 11.9.1–11.9.37.
- Short FJ, Gorton P, Wiseman J, *et al.* (1996) Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim Feed Sci Technol* **59**, 215–221.
- Rutherfurd SM, Bains K & Moughan PJ (2012) Available lysine and digestible amino acid contents of proteinaceous foods of India. *Br J Nutr* **108**, S59–S68.
- Rutherfurd SM, Cui J, Goroncy AK, *et al.* (2015) Dietary protein structure affects endogenous ileal amino acids but not true ileal amino acid digestibility in growing male rats. *J Nutr* **145**, 193–198.
- 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.
- Charan J & Kantharia ND (2013) How to calculate sample size in animal studies? *J Pharmacol Pharmacother* **4**, 303–306.
- Zhang Z, Zhou M, Tang Y, *et al.* (2012) Bioactive compounds in functional buckwheat food. *Food Res Int* **49**, 389–395.
- Lu H, Zhang J, Liu K, *et al.* (2009) Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10 000 years ago. *Proc Natl Acad Sci U S A* **106**, 7367–7372.
- Amadou I, Le G, Amza T, *et al.* (2013) Purification and characterization of foxtail millet-derived peptides with antioxidant and antimicrobial activities. *Food Res Int* **51**, 422–428.
- Wang L, Chen C, Su A, *et al.* (2016) Structural characterization of phenolic compounds and antioxidant activity of the phenolic-rich fraction from defatted adlay (*Coix lachrym-jobi* L. var. ma-yuen Stapf) seed meal. *Food Chem* **196**, 509–517.
- Guo X, Ma Y, Parry J, *et al.* (2011) Phenolics content and antioxidant activity of tartary buckwheat from different locations. *Molecules* **16**, 9850–9867.



35. Zhang L, Liu R & Niu W (2014) Phytochemical and antiproliferative activity of proso millet. *PLOS ONE* **9**, e104058.
36. Chen H, Chung C, Chiang W, *et al.* (2011) Anti-inflammatory effects and chemical study of a flavonoid-enriched fraction from adlay bran. *Food Chem* **126**, 1741–1748.
37. Wielen NVD, Moughan PJ & Mensink M (2017) Amino acid absorption in the large intestine of humans and porcine models. *J Nutr* **147**, 1493–1498.
38. Fuller M (2012) Determination of protein and amino acid digestibility in foods including implications of gut microbial amino acid synthesis. *Br J Nutr* **108**, S238–S246.
39. Nosworthy MG, Neufeld J, Frohlich P, *et al.* (2017) Determination of the protein quality of cooked Canadian pulses. *Food Sci Nutr* **5**, 896–903.
40. 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.
41. 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.