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Values for Digestible Indispensable Amino Acid Score (DIAAS) Determined in Pigs Are Greater for Milk Than for Breakfast Cereals, but DIAAS Values for Individual Ingredients Are Additive in Combined Meals

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ABSTRACT

Background: Breakfast cereals contain low-quality proteins and are often consumed with milk. The digestible indispensable amino acid score (DIAAS) has been used to evaluate protein quality, but it is not known if DIAAS obtained in individual foods is additive in combined meals.

Objectives: The following hypotheses were tested: amino acids (AAs) in milk complement AAs in breakfast cereals to provide a balanced meal, and DIAAS in individual foods is additive in combined meals.

Methods: Six ileal cannulated gilts [body weight mean: 55.6 ± 3.7 (SD) kg] were allotted to a 6 × 6 Latin square with six 7-d periods. Ileal digesta were collected for 9 h on days 6 and 7 of each period. Three diets contained a breakfast cereal (i.e., cornflakes or quick oats) or dry milk as the sole source of AAs. Two additional diets contained a combination of dry milk and cornflakes or quick oats. A nitrogen-free diet was also used, and DIAAS was calculated for cornflakes, quick oats, dry milk, and the 2 combined meals for children aged 6 to 36 mo and individuals older than 36 mo through adulthood. For the combined meals, DIAAS was also predicted from the individual ingredient DIAAS.

Results: Dry milk had greater ($P < 0.05$) DIAAS (123 and 144) than quick oats (57 and 67), but cornflakes had less ($P < 0.05$) DIAAS (16 and 19) than the other ingredients. Both breakfast cereal–dry milk meals had DIAAS close to or greater than 100 for children aged 6 mo to 3 y and for older children, adolescents, and adults, but there were no differences between measured and predicted DIAAS.

Conclusions: The combination of milk and breakfast cereals results in a meal that is balanced in indispensable AAs for humans, and DIAAS obtained from individual ingredients is additive in mixed meals. *J Nutr* 2021;00:1–8.

Keywords: amino acids, breakfast cereals, digestible indispensable amino acid score, milk, protein digestibility

Introduction

Almost 50% of American children consume cereals for breakfast (1). Diets based only on cereals have low protein quality (2), and to meet requirements for amino acids (AAs), higher-quality proteins are needed to complement the protein in cereals to provide a meal that is adequate in all indispensable AAs (3). Breakfast cereals are often consumed in combination with milk, and it is assumed that the combined cereal-milk meal meets requirements for all AAs, although to our knowledge, data to demonstrate this have not been published.

The digestible indispensable amino acid score (DIAAS) may be used to determine protein quality (4). The method is based on AA digestibility determined for each AA at the distal ileum, and the pig has been accepted as an appropriate animal model for estimating AA digestibility if values cannot be determined in humans (4, 5). The DIAAS method allows for calculation of the protein value of individual ingredients and mixed meals consisting of several proteins (4).

Values for DIAAS are based on values for apparent ileal digestibility (AID) of AAs that are corrected for the basal

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Abbreviations used: AA, amino acid; AACF, amino acid in cornflakes; AAM, amino acid in milk; AID, apparent ileal digestibility; AIDCF, apparent ileal digestibility in cornflakes; AIDM, apparent ileal digestibility in milk; AIDP, predicted apparent ileal digestibility; CP, crude protein; DIAAS, digestible indispensable amino acid score; FAO, Food and Agriculture Organization; SID, standardized ileal digestibility.

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endogenous loss of each individual AA, resulting in values defined as standardized ileal digestibility (SID). Values for SID of AAs are additive in mixed diets because these values are independent of basal endogenous losses (6, 7). As a consequence, it is expected that DIAAS obtained for individual food ingredients is additive in a mixed meal, but data to demonstrate this have not been reported. Therefore, the objective of this experiment was to test the hypothesis that milk can be used to complement breakfast cereals to produce a meal that meets requirements for AAs. The second hypothesis was that DIAAS, in combined meals consisting of a breakfast cereal and milk, can be calculated from DIAAS values obtained in individual ingredients.

Methods

The protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois before the experiment was conducted. Pigs that were the offspring of L359 males mated to Camborough females (PIC) were used in the experiment.

Animals and experimental design

Six growing gilts [initial body weight mean: 55.6 ± 3.7 (SD) kg] were surgically equipped with a T-cannula in the distal ileum using procedures adapted from Stein et al. (8). Pigs were allotted to a 6 × 6 Latin square design with 6 diets and six 7-d periods. Pigs were housed individually in pens (1.5 × 2.5 m) in an environmentally controlled room. Each pen had smooth sides and partially slatted floors. A nipple drinker and a feeder were also installed in each pen. At the conclusion of the experiment, pigs had a body weight mean of 85.1 ± 7.7 (SD) kg.

Diets, feeding, and sample collection

Two breakfast cereals (i.e., quick oats and cornflakes) and a lyophilized nonfat dry milk powder were procured (**Supplemental Table 1**). The quick oats and the nonfat dry milk were purchased from Augason Farms Company, and the cornflakes were from Kellogg's Company. Three diets were based on quick oats, cornflakes, or dry milk as the only source of crude protein (CP) and AAs. Two additional diets were based on a combination of milk and quick oats or milk and cornflakes. A nitrogen-free diet was used to determine basal endogenous losses of CP and AAs to enable the calculation of SID of CP and AAs and calculation of DIAAS (**Supplemental Tables 2** and **3**). Vitamins and minerals were included in the diets to meet or exceed current nutrient requirement estimates for growing pigs (9).

The dry milk diet and the nitrogen-free diet were mixed as complete diets with all ingredients included. However, 4 different premixes containing minerals, titanium dioxide, cornstarch, sugar, and salt were prepared and added to the cornflakes diet, the quick oats diet, and the cornflakes or quick oats–dry milk mixtures prior to feeding. All diets, except the nitrogen-free diet and the cornflakes diet, were fed in a liquid form. The diet containing cornflakes as the only source of AAs was prepared by grinding the cornflakes in a food processor (4-Quart Food Processor with LiquiLock Seal System, WFP16SCND; Waring Commercial) and mixing the ground cornflakes with its designated premix. The cornflakes diet contained more sugar because it was necessary to improve palatability of this diet. The cornflakes–dry milk diet was prepared by manually grinding the cornflakes, adding the dry milk (proportion of 2.6:1 cornflakes–dry milk) and the designed premix, and then mixing with water at a ratio of 1:4. The diet containing the dry milk as the only source of AAs was prepared by mixing the whole diet portion with water at a ratio of 1:3.

The quick oats diet was prepared as a porridge by mixing with water at a ratio of 1:3, and this mixture was then heated on a hotplate until boiling, after which time it was removed from the hotplate and left at room temperature to cool. When the porridge had a temperature of ~20°C, it was mixed with the designated premix and offered to the pigs. The quick oats–dry milk diet was prepared the same way, but the dry milk was added when mixing the porridge with the premix (proportion of 2.4:1 quick oats–dry milk). The dry milk was added in different proportions in the mixed diets due to difficulty of mixing the cornflakes with dry milk.

A sample of cornflakes, quick oats, and dry milk and of all diets was collected at the time of diet mixing, and this sample was used for chemical analysis. All diets were fed to 1 pig in each period, and no pig received the same diet more than once during the experiment. There were, therefore, 6 replicate pigs per treatment. Pig weights were recorded at the beginning of each period to calculate feed allowance during the following period, and the amount of feed supplied each day was recorded. All pigs were fed their assigned diets in a daily amount equivalent to 4% of body weight in 2 equal meals that were provided every day at 08:00 and 17:00, and water was available at all times. Feed refusals were recorded daily and zootechnical performance was calculated (**Supplemental Table 4**). Pig weights were also recorded at the conclusion of the experiment. The initial 5 d of each period were considered the adaptation period to the diet, and ileal digesta were collected from 08:00 to 17:00 on days 6 and 7 using standard procedures (8). On the completion of 1 experimental period, animals were deprived of feed overnight, and the following morning, a new experimental diet was offered.

Chemical analyses

At the conclusion of the experiment, ileal digesta samples were thawed and mixed within animal and diet, and a subsample was lyophilized and finely ground prior to analysis. Subsamples of diets were also lyophilized and finely ground before analysis. Samples of all ingredients, diets, and ileal digesta were analyzed for nitrogen by combustion [Method 990.03 (10)] using a LECO FP628 analyzer (LECO Corp.) and for AAs [Method 982.30 E (a, b, c) (10)]. The CP was calculated as nitrogen × 6.25. Dry matter was analyzed in the dry milk and in the diets containing milk as described by Ahn et al. (11), but Method 930.15 (10) was used to analyze dry matter in diets, ingredients, and ileal digesta samples that did not contain milk. Diets and ingredients were analyzed for ash [Method 942.05 (10)], and gross energy was analyzed using an isoperibol bomb calorimeter (Model 6300; Parr Instruments) with benzoic acid as the standard for calibration. Acid-hydrolyzed ether extract was analyzed by acid hydrolysis using 3*N* HCl (AnkomHCl; Ankom Technology) followed by crude fat extraction using petroleum ether (AnkomXT15; Ankom Technology). Cornflakes and quick oats were also analyzed for insoluble dietary fiber and soluble dietary fiber [Method 991.43 (10)] using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology). Starch was analyzed in cornflakes and quick oats using the glucoamylase procedure [Method 979.10 (10)]. Diets and ileal digesta samples were also analyzed for titanium (12).

Calculations

The AID and SID of CP and all AAs in each diet were calculated using published equations (13). The predicted AID of AAs in the diet containing cornflakes and dry milk was calculated using the following equation (6):

$$AIDP = [(AACF \times AIDCF) + (AAM \times AIDM)/(AACF + AAM) \quad (1)$$

where AIDP (%) is the predicted AID for an AA in the mixed diet, and AACF and AAM are the concentrations (%) of that AA contributed by cornflakes and dry milk, respectively, which were calculated by multiplying the concentration of that AA (%) in the ingredient by the proportion (%) of the ingredient in the mixed diet. The determined AID (%) of the AA in cornflakes and dry milk was designated as AIDCF and AIDM, respectively. The predicted AID of CP and the SID of CP and all AAs in the diet containing cornflakes and dry milk were calculated using the same equation. Values for the predicted AID and SID of CP and all AAs in the quick oats–dry milk diet were also calculated using this equation. In the calculations of predicted values for AID and SID in the mixed diets, no effects of period were observed; therefore, values obtained from the same pig for each ingredient were used.

TABLE 1 Apparent ileal digestibility and standardized ileal digestibility of CP and AAs for ingredients fed to growing pigs[1]

	Apparent ileal digestibility					Standardized ileal digestibility				
Item, %	Cornflakes	Quick oats	Dry milk	SEM	*P* value	Cornflakes	Quick oats	Dry milk	SEM	*P* value
n[2]	6	6	6			6	6	6		
CP	55.3[c]	79.4[b]	89.5[a]	1.39	<0.001	98.6[a]	91.9[b]	101.8[a]	1.39	0.007
Indispensable amino acids										
Arg	4.6[b]	86.1[a]	93.1[a]	5.56	<0.001	109.1	94.5	108.4	5.56	0.160
His	68.6[c]	81.7[b]	94.8[a]	1.15	<0.001	87.7[b]	87.5[b]	99.3[a]	1.15	<0.001
Ile	68.7[c]	80.7[b]	91.2[a]	1.25	<0.001	92.5[a]	86.7[b]	95.4[a]	1.25	0.0003
Leu	87.8[b]	83.3[c]	95.2[a]	0.63	<0.001	96.9[b]	88.1[c]	98.6[a]	0.63	<0.001
Lys	− 13.6[c]	75.9[b]	93.1[a]	4.23	<0.001	77.9[b]	82.7[b]	96.3[a]	4.23	0.018
Met	81.0[c]	84.9[b]	96.2[a]	0.98	<0.001	97.7[a]	88.8[b]	98.5[a]	0.98	<0.001
Phe	80.1[c]	84.4[b]	95.2[a]	0.78	<0.001	94.8[b]	88.4[c]	99.3[a]	0.78	<0.001
Thr	49.9[c]	73.7[b]	87.7[a]	2.17	<0.001	93.1[a]	84.7[b]	95.6[a]	2.17	0.003
Trp	66.3[c]	78.8[b]	92.8[a]	2.63	0.0004	91.4[ab]	85.2[b]	96.8[a]	2.63	0.024
Val	63.2[c]	78.5[b]	91.0[a]	1.38	<0.001	94.3[a]	85.9[b]	96.6[a]	1.38	<0.001
Dispensable amino acids										
Ala	76.4[b]	77.1[b]	87.3[a]	1.70	0.029	97.6[a]	87.0[b]	100.0[a]	1.70	0.023
Asp	48.9[c]	79.4[b]	90.9[a]	1.62	<0.001	87.2[b]	86.4[b]	97.6[a]	1.62	0.0002
Cys	62.4[b]	84.3[a]	80.5[a]	2.14	<0.001	92.6[b]	89.6[b]	99.1[a]	2.14	0.013
Glu	84.3[c]	89.3[b]	93.5[a]	0.68	<0.001	95.8[a]	92.3[b]	96.3[a]	0.68	0.002
Ser	69.5[b]	82.9[a]	85.9[a]	1.13	<0.001	95.3[a]	90.1[b]	91.9[ab]	1.13	0.019
Tyr	71.3[c]	82.4[b]	94.7[a]	0.99	<0.001	95.4[b]	88.1[c]	99.0[a]	0.99	<0.001

[1]Values are means and pooled SEMs. Labeled means in a row without a common superscript letter differ, $P < 0.05$. SID values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses of amino acids were calculated from pigs fed the nitrogen-free diet as follows (g/kg DM intake): CP, 16.83; Arg, 0.76; His, 0.18; Ile, 0.32; Leu, 0.49; Lys, 0.38; Met, 0.09; Phe, 0.29; Thr, 0.49; Trp, 0.08; Val, 0.52; Ala, 0.61; Asp, 0.75; Cys, 0.22; Glu, 0.89; Ser, 0.43; Tyr, 0.25. AA, amino acid; AID, apparent ileal digestibility; Ala, alanine; Arg, arginine; Asp, aspartate; CP, crude protein; Cys, cysteine; DM, dry matter; Glu, glutamate; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Ser, serine; SID, standardized ileal digestibility; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.

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

The DIAAS reference ratio for each protein source or mixed diet was calculated using the following equation (14):

$$\begin{aligned}&\text{Digestible indispensable AA reference ratio}\\&\quad = \text{mg digestible indispensable AA content in 1 g protein of food/}\\&\qquad \text{mg of the same dietary indispensable AA in 1 g of the reference protein}\end{aligned} \tag{2}$$

Separate reference ratios were calculated for 2 age groups: children from 6 to 36 mo and for older children, adolescents, and adults (4).

The DIAAS values were also calculated for these age groups as recommended by Food and Agriculture Organization (FAO) (4) using the following equation:

$$\begin{aligned}\text{DIAAS}\,(\%) = 100 \times \text{lowest value of the digestible}\\ \text{indispensable AA reference ratio.}\end{aligned} \tag{3}$$

Statistical analyses

The number of replicates per treatment was based on recommendations from FAO (15). At the conclusion of the experiment, normality of residuals was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures, respectively (SAS Institute). Data were analyzed by ANOVA using the PROC MIXED procedure in SAS. The pig was the experimental unit for all analyses. Diet was the fixed effect, and pig and period were random effects. Treatment means were calculated using the LSMEANS statement in SAS, and when significant, means were separated using the PDIFF option in the MIXED procedure. A *t*-test was used to test the null hypothesis that the difference between the determined and predicted AID or SID of CP and AAs, as well as DIAAS for the mixed diets, was equal to zero. Significance and tendencies were considered at $P < 0.05$ and $0.05 \le P < 0.10$, respectively.

Results

Health of pigs was evaluated daily by the investigators and the farm staff and followed a protocol approved by the Animal Care and Use Committee at the University of Illinois. All pigs remained healthy during the experimental period, and only little feed refusals were observed.

The AID for CP and all AAs was greater ($P < 0.05$) in dry milk than in cornflakes (**Table 1**). Likewise, the AID for CP and all AAs was greater ($P < 0.05$) in dry milk than in quick oats, except for arginine (Arg), cysteine (Cys), and serine (Ser). Cornflakes also had less ($P < 0.05$) AID for CP and all AAs compared with quick oats, except for alanine (Ala). The SID for CP, isoleucine (Ile), methionine (Met), threonine (Thr), valine (Val), Ala, and glutamate (Glu) was greater ($P < 0.05$) in cornflakes and dry milk than in quick oats, and dry milk had greater ($P < 0.05$) SID for histidine (His), leucine (Leu), lysine (Lys), phenylalanine (Phe), aspartate (Asp), Cys, and tyrosine (Tyr) than both cereals. Quick oats had lower ($P < 0.05$) SID of tryptophan (Trp) than dry milk and also had lower ($P < 0.05$) SID of Ser than cornflakes.

Differences between measured and predicted AID in the cornflakes–dry milk diet differed ($P < 0.05$) from zero for CP, Arg, Met, Ala, Asp, and Tyr and tended ($0.05 \le P < 0.10$) to differ from zero for His, Lys, Phe, and Thr (**Table 2**). Differences between measured and predicted values for SID differed ($P < 0.05$) from zero for Ile, Glu, and Ser and tended ($0.05 \le P < 0.10$) to differ from zero for Leu, Val, and Cys. However, for all other AAs, no differences between measured and predicted values for AID and SID of AA in the cornflakes–dry milk diet were observed. For the quick oats–dry milk diet, the AID and SID of Ser tended ($0.05 \le P < 0.10$) to be different between

TABLE 2 Measured and predicted values for AID and SID of CP and AAs in the cornflakes–dry milk meal-based diet fed to growing pigs[1]

	Apparent ileal digestibility				Standardized ileal digestibility			
Item, %	Measured	Predicted	Difference	SEM	Measured	Predicted	Difference	SEM
n[2]	6	6			6	6		
CP	85.1	79.0	6.1**	1.31	98.4	100.8	− 2.4	1.31
Indispensable amino acids								
Arg	88.0	71.1	16.9**	2.92	108.7	108.6	0.1	2.92
His	88.8	86.6	2.2+	1.06	94.1	95.7	− 1.6	1.06
Ile	86.0	85.5	0.5	0.91	91.4	94.6	− 3.2*	0.91
Leu	93.0	92.1	1.0	0.61	96.6	97.9	− 1.3+	0.61
Lys	89.1	86.6	2.5+	1.19	94.3	95.2	− 0.9	1.19
Met	95.0	92.4	2.6**	0.53	98.0	98.3	− 0.3	0.53
Phe	91.9	90.1	1.8+	0.74	96.6	97.8	− 1.2	0.74
Thr	82.1	78.0	4.0+	1.61	92.3	95.0	− 2.7	1.61
Trp	88.5	89.2	− 0.7	1.28	94.2	96.1	− 1.9	1.27
Val	86.2	84.1	2.1	1.13	93.5	96.0	− 2.5+	1.13
Dispensable amino acids								
Ala	86.9	81.5	5.4*	1.68	97.8	98.7	− 0.9	1.68
Asp	83.9	80.1	3.8*	1.36	92.7	94.9	− 2.2	1.36
Cys	74.2	71.5	2.6	2.79	89.1	95.9	− 6.8+	2.79
Glu	90.7	90.6	0.1	0.61	94.1	96.1	− 2.0*	0.61
Ser	81.1	80.8	0.4	1.10	88.5	93.0	− 4.5**	1.10
Tyr	92.1	87.9	4.1**	0.75	97.3	97.9	− 0.7	0.75

[1]Values are means and pooled SEMs. Labeled means in a row differ if *measured compared with predicted, $P \leq 0.05$, or **measured compared with predicted, $P \leq 0.01$, or tend to differ if +measured compared with predicted, $0.05 < P \leq 0.10$. For definitions of abbreviations, see Table 1.
[2]n indicates the number of replicates for each item within each treatment.

measured and predicted values (Table 3), but for CP and all other AAs, no differences between measured and predicted values for AID and SID were observed in the quick oats–dry milk diet.

For children aged 6 mo to 3 y and for older children, adolescents, and adults, dry milk had greater ($P < 0.05$) DIAAS than the other diets, whereas cornflakes had less ($P < 0.05$) DIAAS than the other diets (Table 4). The first limiting AA for both age groups was Lys in cornflakes and quick oats, but there was no limiting AA in dry milk because DIAAS values >100 are not considered limiting. For both age groups, there was no limiting AA for the cornflakes-dry milk combination. Likewise, there was no limiting AA for the quick oats-dry milk combination for individuals older than 36 mo, however, for children from 6 to 36 mo, Lys was the first limiting AA (Table 5). The measured and predicted reference values for cornflakes–dry milk differed ($P < 0.05$) from zero for Ile and tended ($0.05 \leq P < 0.10$) to differ from zero for Leu, sulfur amino acid, and Val. In contrast, no differences between the predicted and the measured reference values for any AA in the quick oats–dry milk combination were observed. Regardless of age group, there were no differences between predicted and measured DIAAS values.

Discussion

The nutrient composition of cornflakes, quick oats, and dry milk was generally within the range of published values (16) for these food ingredients. Cornflakes had lower CP, AA, total dietary fiber, and acid-hydrolyzed ether extract compared with corn (9) and was also very low in Lys, indicating that the cornflakes may have been heat damaged because overheating reduces the concentration of Lys in foods (17). The observation that the AID for Lys in cornflakes was negative and the SID of Lys was the lowest among all AAs also indicates that the cornflakes used in this experiment were heat damaged. However, SID of CP and most AAs, except Lys, in cornflakes were greater than reported for corn (9, 14), which is likely due to processing and to the lower concentration of total dietary fiber in cornflakes compared with corn. It is also possible that the cornflakes being ground prior to feeding may have affected the SID of AAs, but it was necessary to grind cornflakes to make the pigs consume them. The SID of CP and AAs that were measured for quick oats and dry milk were within the range of reported values for dehulled oats and dry skim milk, respectively (3, 9, 14).

The observation that only few differences between the measured and the predicted SID of AAs were observed for the cornflakes–dry milk diet, and none for the quick oats–dry milk diet, demonstrates that SID values in mixed meals can be predicted from individual ingredients as previously demonstrated for other types of diets (6, 7). The reason there were a few differences between measured and predicted values for AID of AAs in the cornflakes–dry milk diet is that values for AID are not always additive in mixed diets if the concentration of AAs in one of the ingredients is less than in the mixed diet (6). The reason no differences between the measured and predicted AID in the quick oats–dry milk diet were observed likely is that the concentration of CP and AAs in the quick oats diet, the dry milk diet, and the quick oats–dry milk diet was not different, and if that is the case, AID values are also expected to be additive in mixed diets (6).

The DIAAS for cornflakes was very low for both age groups, and Lys was the first limiting AA, which is due to the low concentration and the low SID of Lys that was determined for this ingredient. The reason Trp was the second limiting AA in cornflakes is that corn protein is always low in Lys and Trp (18). Lys is also the first limiting AA in whole corn

TABLE 3 Measured and predicted values for AID and SID of CP AAs in the quick oats–dry milk meal-based diet fed to growing pigs[1]

	Apparent ileal digestibility				Standardized ileal digestibility			
Item, %	Measured	Predicted	Difference	SEM	Measured	Predicted	Difference	SEM
n^2	5	5			5	5		
CP	85.0	84.1	0.6	0.69	96.9	96.4	0.2	0.69
Indispensable amino acids								
Arg	87.6	88.5	− 1.2	0.76	98.0	99.1	− 1.4	0.76
His	88.5	88.8	− 0.4	0.65	93.6	93.9	− 0.5	0.65
Ile	87.1	86.6	0.4	0.62	92.1	91.7	0.3	0.62
Leu	89.8	89.9	− 0.2	0.59	93.9	94.0	− 0.2	0.59
Lys	87.3	87.1	− 0.2	0.86	91.8	91.6	− 0.1	0.86
Met	91.8	91.6	0.1	0.56	94.7	94.5	0.1	0.56
Phe	89.1	89.5	− 0.5	0.67	93.1	93.5	− 0.5	0.67
Thr	81.9	81.4	0.3	0.77	91.3	90.7	0.4	0.77
Trp	87.7	86.5	1.0	0.64	92.8	91.6	0.9	0.64
Val	85.9	85.2	0.6	0.61	92.3	91.6	0.5	0.60
Dispensable amino acids								
Ala	81.9	81.1	0.5	1.47	92.7	92.2	0.3	1.47
Asp	85.2	85.0	0.0	0.95	92.0	91.9	− 0.1	0.95
Cys	85.2	83.7	1.2	0.62	92.6	91.6	0.7	0.62
Glu	91.7	91.5	0.1	0.45	94.6	94.4	0.1	0.45
Ser	85.7	84.6	1.0^+	0.46	92.3	91.2	1.0^+	0.46
Tyr	88.7	89.3	− 0.7	0.49	93.7	94.2	− 0.5	0.49

[1]Values are means and pooled SEMs. Labeled means in a row tend to differ if $^+$measured compared with predicted, $0.05 < P \leq 0.10$. For definitions of abbreviations, see Table 1.
[2]*n* indicates the number of replicates for each item within each treatment.

(14), but the reported DIAAS in whole corn is greater than the DIAAS for cornflakes determined in this experiment, which is likely because of the heat damage that reduced the DIAAS of cornflakes. A very low DIAAS for a corn-based breakfast cereal was also reported previously further, indicating that the processing used to prepare breakfast cereals may result in reduced digestibility of Lys (19). The observation that Lys was also the first limiting AA in quick oats is in agreement with data for dehulled oats and oat protein concentrate, and the DIAAS in quick oats is close to the DIAAS for dehulled oats and oat protein concentrate (14, 20, 21). The high DIAAS in dry milk is consistent with data reported by Mathai et al. (3) and by Rutherfurd et al. (19), and this reflects the greater protein quality in milk compared with cereal grains and breakfast cereals produced from cereal grains.

Values for DIAAS in grain-based diets are defined by the Lys concentration and digestibility because Lys is first limiting in all grain products (14). This represents a problem in foods that have undergone early or advanced Maillard reactions during processing. Advanced Maillard reaction products will not be analyzed as Lys, but early Maillard reaction products may revert back to Lys during acid hydrolysis prior to AA analysis (4, 22). The AA analysis, therefore, may overestimate Lys concentration, and therefore also DIAAS, in these ingredients. The observation that the AID of Arg in cornflakes was very low indicates that Arg, which also contains amino groups in the side chain, may also have been heat damaged during processing. It is, however, also possible that the diet-specific endogenous loss of Arg was greater when pigs were fed the cornflakes diet, which also may have contributed to the low AID of Arg.

The DIAAS of almost 100 for children aged 6 to 36 mo and >100 for individuals older than 36 mo indicates that the combination of cornflakes or quick oats and milk provides a balanced ratio of AAs, and if consumed in sufficient quantities, this combination will meet dietary requirements for AAs for those age groups. The reason DIAAS in the cornflakes–dry milk meal was greater than in the quick oats–dry milk meal, despite the greater DIAAS for quick oats compared with cornflakes, is that the inclusion of milk was greater in the cornflakes–dry milk diet than in the quick oats–dry milk diet.

Results of this experiment confirm that the high protein quality in milk can compensate for low protein quality in cereal grains if milk and cereal grains are combined. Because Lys was the AA in least concentration relative to the requirement in both meals, the daily intake of the meal to meet AA requirements is defined by the intake of Lys. If it is assumed that human adults have a daily Lys requirement of 30 mg/kg body weight (23) and that one-third of the daily Lys requirement needs to be satisfied by eating breakfast, an 80-kg adult human will need to consume ∼115 g of the cornflakes-milk meal or 100 g of the quick oats–milk meal to meet the requirement for all AAs.

One advantage of using DIAAS to evaluate protein quality is that values are not truncated to 100, which allows for calculation of complementary effects of high-quality proteins in mixed meals, as demonstrated in this experiment. Because most individuals eat meals that consist of several proteins, it is important that values for protein quality of individual ingredients are additive in mixed meals because that allows for calculation of the protein quality of mixed meals from the quality of individual ingredients. Although fat-free milk powder was used in this experiment, it is likely that results can be extrapolated to diets containing breakfast cereals and full-fat or partially defatted milk products. If anything, fat-containing milk may have slightly greater DIAAS than the nonfat milk used in this experiment because dietary fat increases AA digestibility due to slower gastric emptying (24).

The demonstrated additivity of SID values for most indispensable AAs is in agreement with previous data (6, 7). The observation that among all the indispensable AAs, only the branched-chain AAs in the cornflakes-milk diet tended to not

TABLE 4 DIAAS for the ingredients as measured in growing pigs[1]

Item	Cornflakes	Quick oats	Dry milk	SEM	*P* value
n[2]	6	6	6		
Child (6 mo to 3 y)[3]					
DIAA reference ratio					
His	1.27	0.95	1.40		
Ile	1.19	1.03	1.62		
Leu	2.31	0.96	1.47		
Lys	0.16	0.57	1.38		
SAA	1.32	1.51	1.22		
AAA	1.88	1.45	1.87		
Thr	0.98	0.84	1.29		
Trp	0.55	1.09	1.66		
Val	1.05	1.00	1.44		
DIAAS,[4] %	16[c] (Lys)	57[b] (Lys)	123[a]	1.13	<0.001
Older child, adolescent, adult[5]					
DIAA reference ratio					
His	1.59	1.18	1.75		
Ile	1.27	1.10	1.72		
Leu	2.50	1.03	1.59		
Lys	0.19	0.67	1.64		
SAA	1.56	1.77	1.44		
AAA	2.38	1.84	2.38		
Thr	1.21	1.04	1.61		
Trp	0.71	1.40	2.14		
Val	1.13	1.07	1.55		
DIAAS,[4] %	19[c] (Lys)	67[b] (Lys)	144[a]	1.31	<0.001

[1]Values are means and pooled SEMs. Labeled means in a row without a common superscript letter differ, $P < 0.05$. AAA, aromatic amino acid; DIAA, digestible indispensable amino acid; DIAAS, digestible indispensable amino acid score; SAA, sulfur amino acid. For definitions of the rest of the abbreviations, see Table 1.
[2]*n* indicates the number of replicates for each item within each treatment.
[3]DIAA reference ratios and DIAAS were calculated using the recommended AA scoring pattern for a child (aged 6 mo to 3 y). The indispensable AA reference patterns are expressed as mg AA/g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; SAA, 27; AAA, 52; Thr, 31; Trp, 8.5; Val, 43 (4).
[4]First-limiting AA is in parentheses. A DIAAS value >100 indicates that no AA was limiting.
[5]DIAA reference ratios and DIAAS were calculated using the recommended AA scoring pattern for an older child, adolescent, and adult. The indispensable AA reference patterns are expressed as mg AA/g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; SAA, 23; AAA, 41; Thr, 25; Trp, 6.6; Val, 40 (4).

be different may be a result of the Leu-induced catabolism of Ile and Val that may take place if Leu is present in concentrations greater than the requirement, as is often the case in diets based on corn protein (25).

The present data demonstrate, to our knowledge, for the first time that DIAAS values for individual ingredients are additive in mixed meals. The implication of this observation is that if a database for DIAAS in individual food ingredients can be established, it is possible to predict DIAAS in combinations of different foods. Therefore, it will be beneficial if DIAAS can be determined in more food proteins. Although the principles for additivity of DIAAS in mixed diets demonstrated in this research is believed to be applicable to all types of mixed meals, additional research to demonstrate additivity of DIAAS in other combinations of meals should be conducted.

In some cases, it may not be possible to use DIAAS values for foods that are processed exactly as the foods used in a specific situation, and the question, then, is if DIAAS obtained in foods processed in a different way can be used to calculate DIAAS in a combined meal. To address this question, DIAAS values for the combined meals of cornflakes and milk or quick oats and milk were calculated using published DIAAS values for a corn-based breakfast cereal and cooked rolled oats as well as for skimmed milk powder (3, 19). Results of these calculations indicated that the cornflakes-milk DIAAS based on published values was less than calculated in this experiment (91 and 108 for children aged 6–36 mo and individuals aged >36 mo, respectively, compared with the DIAAS of 101 and 120 obtained in this experiment). For the quick oats–milk diet, the calculated values were 97 and 115 for children aged between 6 and 36 mo and individuals aged >36 mo, respectively, and these values were in very good agreement with the values calculated in this experiment (95 and 113, respectively). It thus appears that under certain circumstances, values for DIAAS in a mixed meal may be calculated using values for slightly different ingredients.

Whereas calculations of DIAAS values for food proteins represent a significant improvement in evaluation of food proteins compared with previously used methods (3, 4), diets for monogastric livestock and companion animals are usually not formulated based on protein scores. Instead, mixed diets for animals are formulated by matching SID values for each indispensable AA obtained in each ingredient and calculated for the mixed diet, to the requirement of each individual AA. This is a more logical and accurate procedure than assigning a protein score to each ingredient. However, because SID values for food proteins are generated in the calculation of DIAAS, it will be possible to use these values if, at a later time, human food protein evaluation will be based directly on SID values for each AA.

In conclusion, data from this experiment demonstrate that regardless of age group, cornflakes or quick oats are low-quality proteins, and individuals consuming only these grains will receive an unbalanced supply of indispensable AAs. However, when younger children, older children, adolescents, and adults

TABLE 5 Measured and predicted values for DIAAS in combined meals of cornflakes–dry milk or quick oats–dry milk fed to growing pigs[1]

Item	Cornflakes–dry milk				Quick oats–dry milk			
	Measured	Predicted	Difference	SEM	Measured	Predicted	Difference	SEM
n[2]	6	6			5	5		
Child (6 mo to 3 y)[3]								
DIAA reference ratio								
His	1.34	1.36	– 0.02	0.02	1.16	1.16	– 0.01	0.01
Ile	1.43	1.48	– 0.05*	0.01	1.31	1.30	0.00	0.01
Leu	1.70	1.73	– 0.02+	0.01	1.20	1.20	0.00	0.01
Lys	1.00	1.01	– 0.01	0.01	0.95	0.95	0.00	0.01
SAA	1.22	1.26	– 0.03+	0.02	1.38	1.38	0.00	0.01
AAA	1.86	1.87	– 0.02	0.01	1.64	1.65	– 0.01	0.01
Thr	1.16	1.20	– 0.03	0.02	1.06	1.05	0.00	0.01
Trp	1.30	1.32	– 0.03	0.02	1.37	1.36	0.01	0.01
Val	1.29	1.32	– 0.04+	0.02	1.21	1.21	0.01	0.01
DIAAS,[4] %	100	101	– 0.96	1.27	95 (Lys)	95 (Lys)	– 0.15	0.89
Older child, adolescent, adult[5]								
DIAA reference ratio								
His	1.68	1.70	– 0.03	0.02	1.45	1.45	– 0.01	0.01
Ile	1.53	1.58	– 0.06*	0.01	1.40	1.39	0.00	0.01
Leu	1.84	1.87	– 0.03+	0.01	1.29	1.30	0.00	0.01
Lys	1.19	1.20	– 0.01	0.02	1.13	1.13	0.00	0.01
SAA	1.44	1.47	– 0.04+	0.02	1.63	1.62	0.00	0.01
AAA	2.35	2.38	– 0.02	0.02	2.08	2.09	– 0.01	0.01
Thr	1.44	1.48	– 0.04	0.03	1.31	1.31	0.00	0.01
Trp	1.67	1.70	– 0.03	0.02	1.76	1.75	0.02	0.01
Val	1.38	1.42	– 0.04+	0.02	1.30	1.30	0.01	0.01
DIAAS,[4] %	119	120	– 1.14	1.50	113	113	– 0.17	1.06

[1]Values are means and pooled SEMs. Labeled means in a row differ if *measured compared with predicted, $P \leq 0.05$, or tend to differ if +measured compared with predicted, $0.05 < P \leq 0.10$. AAA, aromatic amino acid; DIAA, digestible indispensable amino acid; DIAAS, digestible indispensable amino acid scores; SAA, sulfur amino acid. For definitions of the rest of the abbreviations, see Table 1.

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

[3]DIAA reference ratios and DIAAS were calculated using the recommended AA scoring pattern for a child (aged 6 mo to 3 y). The indispensable AA reference patterns are expressed as mg AA/g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; SAA, 27; AAA, 52; Thr, 31; Trp, 8.5; Val, 43 (4).

[4]First-limiting AA is in parentheses. A DIAAS value ≥100 indicates that no AA was limiting.

[5]DIAA reference ratios and DIAAS were calculated using the recommended AA scoring pattern for an older child, adolescent, and adult. The indispensable AA reference patterns are expressed as mg AA/g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; SAA, 23; AAA, 41; Thr, 25; Trp, 6.6; Val, 40 (4).

consume a combination of breakfast cereals and milk, they will consume a meal with a DIAAS close to or >100, indicating that the high protein quality of milk complements the low protein quality of cereals to generate a diet that is balanced in indispensable AAs. Results also demonstrated that DIAAS obtained for individual ingredients is additive in mixed meals when fed to growing pigs, and the implication of this observation is that DIAAS in combined meals can be calculated from values obtained in individual ingredients.

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