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Non Ruminant Nutrition

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The direct and difference procedures result in similar estimates for amino acid digestibility in feed ingredients fed to growing pigs

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Abstract

An experiment was conducted to test the hypothesis that values for standardized ileal digestibility (**SID**) of amino acids (**AA**) in corn, wheat, and wheat middlings obtained using the direct procedure are not different from values obtained using the difference procedure. Sixteen ileal-cannulated barrows (initial BW: 69.5 ± 5.0 kg) were allotted to a replicated 8 × 4 Youden Square design with 8 diets and 4 periods. Each period consisted of 5 d of adaptation to the diet and 2 d of collection of ileal digesta. Four diets were based on soybean meal (**SBM**), corn, wheat, or wheat middlings as the only AA-containing ingredients. Three additional diets were based on a mixture of SBM and corn, SBM and wheat, or SBM and wheat middlings, and an N-free diet was also used. The apparent ileal digestibility (**AID**) and the SID of crude protein (**CP**) and AA for the 4 diets containing SBM, corn, wheat, or wheat middlings as the sole source of AA were calculated using the direct procedure. The AID and SID of CP and AA for the 3 mixed diets containing SBM and corn, wheat, or wheat middlings were also calculated and the contribution of digestible AA from SBM was subtracted from the AID or SID values for the diets. The AID or SID of AA in corn, wheat, and wheat middlings were subsequently calculated by difference. Results indicated that the AID values for a few AA were lower ($P < 0.05$) if the direct procedure was used instead of the difference procedure, regardless of ingredient. The AID of Trp was greater in corn and wheat middlings, and the SID of Trp in corn and wheat middlings tended to be greater, if the direct procedure rather than the difference procedure was used, but that was not the case for wheat (interaction, $P < 0.05$ and $P < 0.10$, respectively). However, for all other indispensable AA, and for most of the dispensable AA, the SID of AA in corn, wheat, and wheat middlings was not different between the difference procedure and the direct procedure. Therefore, values for SID of AA in cereal grains and fiber-rich ingredients may be determined using either the direct or the difference procedure.

Key words: amino acids, difference procedure, digestibility, direct procedure, soybean meal, wheat middlings

Introduction

Values for the standardized ileal digestibility (**SID**) of amino acids (**AA**) are used in diet formulation for pigs, because these values are independent of basal ileal endogenous losses of AA, and SID values are, therefore, additive in mixed diets (Stein et al., 2005; NRC, 2012; Kong and Adeola, 2014). Values for SID of AA may be determined using the direct or difference procedure (Kong and Adeola, 2014; Stein, 2017). If the direct procedure is used, the test ingredient provides all AA to the diet and the SID of AA in the diet also represents the SID of

Abbreviations

AA	amino acids
AID	apparent ileal digestibility
CP	crude protein
DM	dry matter
SBM	soybean meal
SID	standardized ileal digestibility

AA in the test ingredient. However, if the difference procedure is used, a basal diet in which a basal AA-containing ingredient provides all the AA is formulated, and a second diet in which both the test ingredient and the basal ingredient are included is also formulated. The SID of AA in both diets is determined, and the SID of AA in the basal ingredient is equivalent to the SID of AA in the basal diet. The contribution of digestible AA from the basal ingredient to the diet that contains the test ingredient is calculated and the SID of AA in the test ingredient is subsequently calculated by difference.

Because the test ingredient is the sole source of AA in the diet if the direct procedure is used, diets with very low concentrations of AA are sometimes produced (Pedersen et al., 2007; Rojas and Stein, 2015; Casas and Stein, 2017). Pigs fed such a diet for a long period may have reduced growth. Some ingredients with high concentrations of fiber or anti-nutritional factors may be impossible to use as the only AA-containing ingredient, and some ingredients have poor palatability if used in large quantities. There is, therefore, often a need for mixing the test ingredient with another ingredient and calculating the SID of AA using the difference procedure, but the difference procedure usually results in greater standard errors associated with the means for the SID of AA compared with using the direct procedure (Almeida et al., 2013; Sulabo et al., 2013a). However, to our knowledge, data that compare the direct and difference procedures to calculate SID of AA for the same ingredients have not been reported. Therefore, the objective of this experiment was to test the hypothesis that values for SID of AA in cereal grains and fiber-rich ingredients obtained using the direct procedure are not different from values obtained using the difference procedure.

Materials and Methods

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. Pigs that were the offspring of Line 359 boars mated to Camborough sows (Pig Improvement Company, Hendersonville, TN) were used.

Sixteen growing barrows (initial BW: 69.5 ± 5.0 kg) that had a T-cannula installed in the distal ileum were allotted to a replicated 8 × 4 Youden Square design with 8 diets and four 7-d periods in each square. To avoid potential carry-over effects from the Youden square, pig allotments were determined using the Balanced Latin Square Allotment Program (Kim and Stein, 2009). There were 2 pigs per diet in each period for a total of 8 observations per treatment. All pigs had been used in a different experiment from 25 to 55 kg and were fed a common grower diet for 14 d before being allotted to diets in this experiment. Pigs were placed in individual pens (1.2 × 1.5 m) that were equipped with a self-feeder, a nipple waterer, smooth sidewalls, and a slatted tribar floor.

Four diets were based on soybean meal (**SBM**), corn, wheat, or wheat middlings with each ingredient providing all AA in one diet (Table 1). Three additional diets were based on a mixture of SBM and corn, SBM and wheat, or SBM and wheat middlings, and an N-free diet was included in the experiment as well (Tables 2 and 3). Thus, a total of 8 diets were formulated. Vitamins and minerals were included in all diets to meet or exceed the estimated nutrient requirements for growing pigs (NRC, 2012). All diets also contained 0.40% chromic oxide as an indigestible marker. Pigs were limit fed to 3 times their estimated energy requirement for maintenance (i.e., 197 kcal/kg $BW^{0.60}$; NRC, 2012), but throughout the experiment, pigs had free access to water. The initial 5 d of each period was considered the adaptation period, whereas ileal digesta were collected for 8 h on d 6 and 7 of each period because 5 d of adaptation followed by 2 d of collection of ileal digesta results in the most accurate estimates for ileal digestibility of AA (Kim et al., 2020). A 225-mL plastic bag was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Bags were removed every 30 min, or whenever full, and replaced with a new bag. Ileal digesta were stored at −20 °C immediately after collection.

At the conclusion of the experiment, ileal digesta were thawed and mixed within animal and diet, and a subsample was collected for analysis. Samples of all diets and of each of the AA-containing ingredients were also collected. Ileal digesta were lyophilized and finely ground before analysis. Samples of diets, ileal digesta, and ingredients were analyzed for crude protein (**CP**; Method 990.03; AOAC Int., 2007) and dry matter (**DM**; Method 930.15; AOAC Int., 2007). These samples were also analyzed for AA (Method 982.30 E [a, b, c]; AOAC Int., 2007), and diets and ileal digesta samples were analyzed for chromium (Method 990.08; AOAC Int., 2007). Diets and ingredients were also analyzed for insoluble and soluble dietary fiber according to Method 991.43 (AOAC Int., 2007) using the Ankom[TDF] Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). The concentrations of total dietary fiber were calculated as the sum of insoluble and soluble dietary fiber.

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

Item	Soybean meal	Corn	Wheat	Wheat middlings
DM, %	90.44	88.30	91.09	91.24
CP, %	41.89	6.18	8.47	14.85
Total dietary fiber, %	18.60	7.10	9.60	40.30
Indispensable AA, %				
Arg	3.33	0.33	0.50	1.02
His	1.19	0.20	0.23	0.41
Ile	2.24	0.26	0.37	0.50
Leu	3.61	0.82	0.68	0.93
Lys	2.92	0.25	0.36	0.64
Met	0.65	0.15	0.17	0.22
Phe	2.44	0.35	0.47	0.61
Thr	1.81	0.26	0.31	0.49
Trp	0.65	0.05	0.12	0.17
Val	2.28	0.34	0.46	0.73
Dispensable AA, %				
Ala	1.99	0.51	0.39	0.72
Asp	5.28	0.51	0.59	1.08
Cys	0.68	0.17	0.25	0.34
Glu	8.33	1.27	2.64	2.70
Gly	1.95	0.29	0.43	0.81
Pro	2.20	0.61	0.84	0.86
Ser	2.03	0.33	0.43	0.55

Table 2. Composition (as-is basis) of experimental diets

Ingredient, %	SBM	Corn	Wheat	Wheat middlings	Corn–SBM	Wheat–SBM	Wheat middlings–SBM	N-free
Soybean meal	34.00	—	—	—	23.50	21.00	22.00	—
Corn	—	95.30	—	—	72.10	—	—	—
Wheat	—	—	95.65	—	—	74.85	—	—
Wheat middlings	—	—	—	42.00	—	—	41.00	—
Limestone	0.60	0.65	1.10	1.20	0.75	1.05	1.20	0.45
Dicalcium phosphate	0.80	1.10	0.30	0.20	0.70	0.15	—	1.60
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	4.00
Vitamin and mineral premix[1]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Cornstarch	51.65	—	—	43.65	—	—	32.85	68.50
Sugar	10.00	—	—	10.00	—	—	—	20.00
Magnesium oxide	—	—	—	—	—	—	—	0.10
Potassium carbonate	—	—	—	—	—	—	—	0.40
Solka floc	—	—	—	—	—	—	—	4.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]The vitamin–micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 3. Analyzed composition (as-is basis) of experimental diets

Item	SBM	Corn	Wheat	Wheat middlings	Corn–SBM	Wheat–SBM	Wheat middlings–SBM	N-free
DM, %	91.94	87.47	90.21	92.78	87.95	91.28	92.45	95.00
CP, %	14.51	5.97	8.10	5.64	14.43	14.95	14.77	0.64
Total dietary fiber, %	7.10	8.10	13.60	19.20	13.40	13.00	23.60	16.70
Indispensable AA, %								
Arg	1.04	0.32	0.47	0.41	0.97	1.01	1.13	0.01
His	0.39	0.19	0.22	0.18	0.42	0.41	0.43	0.00
Ile	0.72	0.25	0.34	0.21	0.69	0.69	0.68	0.02
Leu	1.18	0.76	0.63	0.41	1.43	1.21	1.18	0.03
Lys	0.95	0.25	0.32	0.28	0.85	0.83	0.91	0.02
Met	0.20	0.14	0.15	0.10	0.24	0.23	0.22	0.02
Phe	0.78	0.32	0.43	0.26	0.81	0.81	0.78	0.02
Thr	0.59	0.25	0.28	0.21	0.60	0.58	0.60	0.01
Trp	0.24	0.04	0.10	0.08	0.17	0.21	0.16	0.01
Val	0.74	0.33	0.42	0.31	0.77	0.78	0.80	0.02
Dispensable AA, %								
Ala	0.66	0.49	0.36	0.32	0.84	0.68	0.74	0.02
Asp	1.70	0.49	0.54	0.47	1.55	1.44	1.57	0.04
Cys	0.22	0.16	0.23	0.14	0.27	0.30	0.29	0.02
Glu	2.70	1.18	2.45	1.17	2.83	3.55	2.94	0.04
Gly	0.64	0.28	0.40	0.35	0.65	0.70	0.77	0.01
Pro	0.72	0.56	0.78	0.36	0.94	1.05	0.84	0.02
Ser	0.66	0.30	0.39	0.25	0.69	0.71	0.68	0.01

The apparent ileal digestibility (**AID**) and the SID of CP and AA were calculated for all diets except the N-free diet, and basal endogenous losses of CP and AA were calculated from pigs fed the N-free diet (Stein et al., 2007). Values calculated for the 4 diets containing SBM, corn, wheat, or wheat middlings as the sole source of AA also represent the values for each ingredient, and these values represented the AID and SID of AA calculated by the direct procedure. For the 3 diets that contained both SBM and corn, SBM and wheat, or SBM and wheat middlings, the contribution of digestible AA from SBM was subtracted from the AID or SID values for the diets, and the AID or SID of AA in corn, wheat, and wheat middlings were calculated by difference (Kong and Adeola, 2014).

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Normality of residuals and outliers was tested using the UNIVARIATE procedure of SAS. Means that deviated from the treatment mean by more than 3 times the interquartile range were considered outliers. Data for AID and SID of CP and AA were first analyzed using a model that included the diet as a fixed effect and pig, period, and block as random effects. Data were subsequently analyzed following a 3 × 2 factorial design with 3 ingredients (corn, wheat, and wheat middlings) and 2 procedures (direct or difference). The model included ingredient, procedure, and ingredient × procedure as fixed effects, and pig, period, and block as random effects. Pig was the experimental unit for all analyses and differences were considered significant at $P < 0.05$, whereas $0.05 \leq P < 0.10$ was considered a tendency.

Results

Pigs readily consumed their assigned diets and remained healthy throughout the experiment. There was no difference for the AID of most AA between the SBM diet and the diet based on corn and SBM (Table 4). However, the AID of CP and most AA was less ($P < 0.01$) in diets based on corn, wheat, wheat middlings, SBM and wheat, or SBM and wheat middlings compared with the SBM diet and the diet based on corn and SBM. The SID of CP and most AA was not different among the SBM diet, the corn diet, and the corn–SBM diet (Table 5), but the SID of CP and most AA in these diets was greater ($P < 0.01$) than in diets containing wheat or wheat middlings.

The AID of CP was greater in corn than in wheat and wheat middlings if the direct procedure was used in calculations, but if the difference procedure was used, there was no difference between corn and wheat for the AID of CP (interaction, $P < 0.05$; Table 6). The AID of Trp was not different between corn and wheat, if the direct procedure was used, but if AID was calculated using the difference procedure, the AID of Trp was greater in wheat than in corn (interaction, $P < 0.05$). Values for the AID of Met, Thr, Val, Ala, Gly, and Pro were greater ($P < 0.05$) if calculated using the difference procedure rather than the direct procedure, and values for the AID of Ile and Lys calculated with the difference procedure tended ($P < 0.10$) to be greater than values calculated using the direct procedure. However, for the AID of all other AA, no difference between the direct and the difference procedure was observed.

No interactions between ingredient and procedure were observed for the SID of CP and AA (Table 7), but the SID of Trp tended to decrease in corn and wheat middlings if calculated using the difference procedure, but that was not the case for wheat (interaction, $P = 0.087$). However, the SID of CP and all other indispensable AA was not different between the two procedures. The SID of Pro was greater ($P < 0.05$) and the SID of Glu tended to be greater ($P = 0.097$) if the direct procedure rather than the difference procedure was used, but for all other dispensable AA, no difference between the 2 procedures was observed.

Discussion

The analyzed AA composition of corn, SBM, wheat, and wheat middlings was within the range of reported values (NRC, 2012; Blas et al., 2016; Rostagno et al., 2017). Except for Lys, the AID and SID of CP and AA in SBM was also in close agreement with published data (NRC, 2012; Blas et al., 2016; Oliveira and Stein, 2016). However, the AID and SID of AA in corn was slightly greater than data reported by NRC (2012) and Blas et al. (2016), but the AID and SID of AA in wheat and wheat middlings were within the range of published data (NRC, 2012; Casas and Stein, 2017; McGhee and Stein, 2018). The basal endogenous losses of AA determined in this experiment also are within the range of values determined previously (Park et al., 2013).

In digestibility experiments, the AID and SID of AA in feed ingredients are determined either by the direct or the difference procedure. If the digestibility of AA is determined by the difference procedure, an extra diet with a basal ingredient is required, and the test diet is formulated based on the basal ingredient and the test feed ingredient (Kong and Adeola, 2014).

Table 4. Apparent ileal digestibility (%) of CP and AA in experimental diets calculated using the direct procedure[1,2]

Item, %	SBM	Corn	Wheat	Wheat middlings	Corn–SBM	Wheat–SBM	Wheat middlings–SBM	Pooled SEM	P-values
CP	80.7[a]	73.9[b]	68.6[c]	45.1[d]	80.4[a]	77.02[ab]	73.8[b]	1.66	<0.001
Indispensable AA									
Arg	91.7[a]	85.1[b]	75.1[c]	74.8[c]	90.8[a]	87.3[b]	86.4[b]	1.23	<0.001
His	88.2[a]	85.4[abc]	76.8[d]	71.3[e]	87.6[ab]	84.5[ab]	82.9[c]	1.35	<0.001
Ile	87.3[a]	83.6[ab]	76.0[c]	62.5[d]	87.1[a]	83.4[ab]	82.5[b]	1.91	<0.001
Leu	86.2[ab]	88.0[a]	78.3[d]	66.7[e]	87.9[a]	83.2[bc]	81.8[cd]	1.50	<0.001
Lys	85.3[a]	75.6[b]	62.3[c]	53.6[d]	83.9[a]	78.8[b]	78.9[b]	2.10	<0.001
Met	88.7[ab]	88.9[a]	81.1[d]	69.9[e]	90.4[a]	85.1[bc]	83.9[cd]	1.46	<0.001
Phe	86.4[a]	85.2[ab]	79.9[c]	67.2[d]	86.8[a]	83.9[ab]	81.9[bc]	1.30	<0.001
Thr	80.6[a]	72.0[c]	64.1[d]	46.0[e]	80.3[ab]	75.2[abc]	74.4[bc]	2.35	<0.001
Trp	87.8[a]	65.6[d]	74.4[c]	63.2[d]	82.2[ab]	81.5[b]	73.3[c]	2.15	<0.001
Val	83.2[a]	78.7[ab]	71.5[c]	58.4[d]	83.4[a]	78.8[ab]	77.6[b]	1.84	<0.001
Dispensable AA									
Ala	80.9[a]	84.5[a]	64.6[c]	52.8[d]	83.9[a]	75.4[b]	74.8[b]	1.65	<0.001
Asp	84.2[a]	80.9[ab]	66.5[c]	60.6[d]	83.5[ab]	78.7[b]	78.9[b]	2.03	<0.001
Cys	78.4[ab]	79.4[a]	78.9[ab]	66.8[c]	79.7[a]	77.4[ab]	74.5[b]	1.90	<0.001
Glu	88.3[a]	88.6[a]	89.7[a]	78.9[c]	87.4[ab]	88.9[a]	84.9[b]	1.21	<0.001
Gly	75.4[a]	61.8[cd]	60.4[d]	37.3[e]	71.8[ab]	72.2[ab]	68.3[bc]	2.45	<0.001
Pro	83.2[ab]	80.9[b]	87.0[ab]	59.9[c]	91.0[a]	93.2[a]	87.1[ab]	4.57	0.007
Ser	84.8[a]	80.6[abc]	76.6[c]	60.7[d]	84.4[a]	81.4[ab]	78.8[bc]	1.67	<0.01

[a–e]Means within a column lacking a common superscript letter are different ($P < 0.05$).

[1]Data are least squares means of 7 or 8 observations per treatment.

Table 5. Standardized ileal digestibility (%) of CP and AA in experimental diets calculated using the direct procedure[1,2]

Item, %	SBM	Corn	Wheat	Wheat middlings	Corn–SBM	Wheat–SBM	Wheat middlings–SBM	Pooled SEM	P-values
CP	87.1[ab]	88.6[a]	79.9[c]	61.7[d]	86.6[ab]	83.2[bc]	80.1[c]	1.66	<0.001
Indispensable AA									
Arg	94.1[a]	93.3[a]	80.6[d]	79.8[d]	93.1[ab]	89.6[bc]	88.3[c]	1.26	<0.001
His	90.8[a]	90.5[ab]	81.4[d]	77.0[e]	89.8[ab]	87.0[bc]	85.4[c]	1.35	<0.001
Ile	89.6[a]	90.0[a]	80.9[c]	70.6[d]	89.5[ab]	85.9[ab]	85.0[bc]	1.91	<0.001
Leu	88.6[ab]	91.7[a]	82.8[c]	73.8[d]	89.9[a]	85.6[bc]	84.3[c]	1.50	<0.001
Lys	87.5[a]	83.8[abc]	68.9[d]	61.4[e]	86.4[ab]	81.4[bc]	81.4[c]	2.18	<0.001
Met	90.9[a]	91.9[a]	84.0[b]	74.5[c]	92.2[a]	87.0[b]	85.9[b]	1.46	<0.001
Phe	88.7[ab]	90.7[a]	84.1[c]	74.3d	89.0[ab]	86.2[bc]	84.3[c]	1.30	<0.001
Thr	86.6[a]	85.5[ab]	76.6[c]	63.0[d]	85.9[ab]	81.3[abc]	80.3[bc]	2.35	<0.001
Trp	91.0[a]	84.2[b]	82.0[bc]	73.1[d]	86.6[ab]	85.2[ab]	78.2[cd]	2.15	<0.001
Val	87.3[a]	87.6[a]	78.7[b]	68.3[c]	87.2[a]	82.7[ab]	81.1[b]	1.84	<0.001
Dispensable AA									
Ala	86.1[b]	91.2[a]	74.0[d]	63.7[e]	87.8[ab]	80.5[c]	79.5[c]	1.65	<0.001
Asp	86.8[ab]	89.4[a]	74.4[d]	69.9[d]	86.2[abc]	81.7[bc]	81.7[c]	2.03	<0.001
Cys	83.3[a]	85.9[a]	83.6[a]	74.6[c]	83.6[a]	80.9[ab]	78.3[bc]	1.90	0.001
Glu	90.2[ab]	92.9[a]	91.8[ab]	83.5[d]	89.2[bc]	90.4[ab]	86.8[c]	1.21	0.001
Gly	89.5[ab]	92.5[a]	82.5[c]	63.3[d]	85.1[bc]	85.0[abc]	80.1[c]	2.45	<0.001
Pro	116.4[ab]	121.6[ab]	117.1[ab]	126.9[a]	115.3[b]	115.8[ab]	115.7[ab]	4.57	0.371
Ser	89.5[a]	90.4[a]	84.4[bc]	73.1[d]	88.6[ab]	85.7[abc]	83.4[c]	1.67	<0.001

[a–e]Means within a column lacking a common superscript letter are different (P < 0.05).

[1]Data are least squares means of 7 or 8 observations per treatment.

[2]Values for standardized ileal digestibility were calculated by correcting AID values for basal endogenous losses. Basal endogenous losses were determine using pigs fed the N-free diet as (g/kg DM intake): CP, 10.10; Arg, 0.32; His, 0.11 Ile, 0.18; Leu, 0.32; Lys, 0.24; Met, 0.05; Phe, 0.20; Thr, 0.39; Trp, 0.09; Val 0.33; Ala, 0.38; Asp, 0.47; Cys, 0.12; Glu, 0.58; Gly, 0.98; Pro, 2.60; and Ser, 0.34.

Table 6. Apparent ileal digestibility of CP and AA in corn, wheat, and wheat middlings calculated using the direct or the difference procedure[1]

	Direct procedure			Difference procedure				P-value		
Item, %	Corn	Wheat	Wheat middlings	Corn	Wheat	Wheat middlings	Pooled SEM	Ingredient	Procedure	Ingredient × procedure
CP	73.9[ab]	68.6[bc]	45.1[d]	79.6[a]	71.8[abc]	63.3[c]	2.94	<0.001	0.001	0.034
Indispensable AA										
Arg	85.1	75.1	74.8	86.5	78.2	76.0	2.21	<0.001	0.159	0.896
His	85.4	76.8	71.3	85.8	78.6	74.2	2.11	<0.001	0.191	0.815
Ile	83.6	76.0	62.5	85.4	75.8	69.8	2.87	<0.001	0.085	0.420
Leu	88.0	78.3	66.7	89.9	78.3	72.1	2.45	<0.001	0.152	0.538
Lys	75.6	62.3	53.6	79.1	63.6	63.7	3.65	<0.001	0.096	0.432
Met	88.9	81.1	69.9	92.8	81.3	76.4	2.15	<0.001	0.023	0.263
Phe	85.2	79.9	67.2	88.2	80.5	72.7	2.45	<0.001	0.164	0.611
Thr	72.0	64.1	46.0	79.1	65.9	61.6	3.47	<0.001	0.005	0.151
Trp	65.6[abc]	74.4[a]	63.2[bc]	58.7[c]	72.5[ab]	43.4[d]	3.56	<0.001	0.003	0.045
Val	78.7	71.5	58.4	83.2	72.1	67.6	2.71	<0.001	0.022	0.286
Dispensable AA										
Ala	84.5	64.6	52.8	87.7	67.6	65.8	2.62	<0.001	0.005	0.104
Asp	80.9	66.5	60.6	80.5	65.3	64.2	3.87	<0.001	0.761	0.739
Cys	79.4	78.9	66.8	80.6	75.7	69.4	2.45	<0.001	0.587	0.486
Glu	88.6	89.7	78.9	84.9	88.9	78.9	2.35	0.001	0.615	0.702
Gly	61.8	60.4	37.3	64.0	68.3	59.2	4.87	0.003	0.012	0.122
Pro	80.9	87.0	59.9	99.8	100.5	91.7	5.45	0.001	<0.001	0.147
Ser	80.6	76.6	60.7	82.8	76.5	66.5	2.80	<0.001	0.178	0.556

[a–d]Means within a column lacking a common superscript letter are different (P < 0.05).

[1]Data are least-squares means of 7 or 8 observations per treatment.

Table 7. Standardized ileal digestibility of CP and AA in corn, wheat, and wheat middlings calculated using the direct or the difference procedure[1]

	Direct procedure			Difference procedure				P-value		
Item, %	Corn	Wheat	Wheat middlings	Corn	Wheat	Wheat middlings	Pooled SEM	Ingredient	Procedure	Ingredient × procedure
CP	88.6	79.9	61.7	85.3	77.7	69.5	2.94	<0.001	0.761	0.119
Indispensable AA										
Arg	93.3	80.6	79.8	89.4	80.9	77.9	2.21	<0.001	0.349	0.633
His	90.5	81.4	77.0	87.5	80.9	76.3	2.11	<0.001	0.641	0.806
Ile	90.0	80.9	70.6	87.8	78.4	72.7	2.87	<0.001	0.908	0.678
Leu	91.7	82.8	73.8	91.1	80.5	74.6	2.45	<0.001	0.924	0.825
Lys	83.8	68.9	61.4	82.1	66.9	66.4	3.65	<0.001	0.882	0.522
Met	91.9	84.0	74.5	93.9	82.9	78.0	2.15	<0.001	0.302	0.446
Phe	90.7	84.1	74.3	89.9	82.6	75.1	2.45	<0.001	0.738	0.893
Thr	85.5	76.6	63.0	83.9	72.1	67.4	3.47	<0.001	0.964	0.440
Trp	84.2	82.0	73.1	67.9	76.8	51.8	3.56	<0.001	<0.001	0.087
Val	87.6	78.7	68.3	86.3	75.6	71.00	2.71	<0.001	0.995	0.556
Dispensable AA										
Ala	91.2	74.0	63.7	90.0	72.4	69.7	2.62	<0.001	0.630	0.270
Asp	89.4	74.4	69.9	83.6	68.4	67.6	3.87	<0.001	0.208	0.862
Cys	85.9	83.6	74.6	82.9	78.3	71.9	2.45	0.001	0.182	0.847
Glu	92.9	91.8	83.5	86.4	89.9	80.5	2.35	0.001	0.097	0.585
Gly	92.5	82.5	63.3	75.5	79.4	67.9	4.87	0.001	0.206	0.087
Pro	121.6	117.1	126.9	113.6	115.6	114.0	5.45	0.622	0.045	0.421
Ser	90.4	84.4	73.1	86.3	80.3	70.8	2.80	<0.001	0.206	0.925

[1]Values are means for 7 or 8 observations per treatment.

The advantage of this procedure is that the test diet can provide the AA at the requirement of the animal, but because all errors in measurements will be attributed to the test ingredient, the most accurate results with this procedure is obtained the greater the proportion of AA in the diet that originates from the test ingredient is. As a consequence, results obtained with the difference procedure are usually associated with greater standard errors of means than if the direct procedure is used (Almeida et al., 2013; Sulabo et al., 2013a), which reduces the power of the experiment.

If the direct procedure is used, the test ingredient provides all AA in the diet, and this is an easy procedure that works well if AID and SID of AA in high-protein ingredients are determined because diets can be formulated to contain all AA in required quantities by only using the test ingredient. This procedure has, therefore, been used to generate AID and SID values in SBM, canola meal, sunflower meal, peanut meal, and many other high-protein ingredients (Almeida et al., 2014; Li et al., 2014; Maison and Stein, 2014; Sotak-Peper et al., 2017). The direct procedure may also be used to determine AA digestibility in cereal grains, but in that case, diets are formulated to be deficient in AA because even if 95% to 97% of the test diet consists of the test ingredient, AA requirements of pigs cannot be met. However, despite providing AA below the requirement, the direct procedure has been used to determine AID and SID of AA in most cereal grains (Pedersen et al., 2007; Cervantes-Pahm et al., 2014; Rosenfelder et al., 2015; Strang et al., 2016; 2017; McGhee and Stein, 2018), and it is assumed that feeding AA below the requirement does not affect values for AID and SID of AA. However, to our knowledge, this assumption has not been tested, but results of the present experiment indicate that values for AID and SID of AA in cereal grains are not impacted by the low AA concentration in the test diet because values for AID and SID of AA in corn and wheat were not different between the direct and the difference procedure. The lower standard errors of means associated with the use of the direct procedure compared with the difference procedure gives support to the continued use of the direct procedure to determine AID and SID of AA in cereal grains. Because no basal diet is required if the direct procedure is used, it is also less costly to use this procedure compared with the difference procedure.

To compensate for the low AA concentration in diets containing cereal grains as the only source of AA, a mixture of AA is sometimes provided during the adaptation period, but not during the collection period (Pedersen et al., 2007; Casas and Stein, 2017). This will result in pigs having a more normal growth rate during the experiment, and recent data indicate that because crystalline AA are 100% absorbed from the small intestine, addition of crystalline AA to test diets does not impact results or AID and SID of AA (Oliveira et al., 2018). Based on the results of this experiment as well as previous data, it is, therefore, concluded that the direct procedure may be used to determine AID and SID values in cereal grains.

In experiments in which AID and SID of AA in grain co-products such as distillers-dried grains with solubles, wheat middlings, or rice bran are determined, the high-fiber concentration in those ingredients does not allow for formulating diets that contain AA at the requirement if the direct procedure is used. Because of the high-fiber concentration in these ingredients it is usually necessary to restrict inclusion to 40% to 60% of the diet, which results in test diets with a relatively low concentration of AA (Casas and Stein, 2017; Espinosa et al., 2018). The high-fiber concentration in these ingredients may also result in stoppage of the cannula if greater inclusion rates are used, which results in a less representative collection of digesta. A further complication with high-fiber cereal co-products is that they sometimes have low palatability if used at a high inclusion rate in the diet, but if the difference procedure is used, diets with greater palatability may be formulated (Casas et al., 2015). However, the results of this experiment demonstrate that for wheat middlings, there are only minor differences between the difference procedure and the direct procedure, indicating that SID values are not influenced by the procedure used. Therefore, it is likely that this conclusion may be extrapolated to other high-fiber grain co-products, but research to verify this hypothesis is needed. Nevertheless, as is the case for cereal grains, the reduced standard errors for AID and SID of AA obtained with the direct procedure compared with the difference procedure indicate that fewer replicates are needed to obtain a certain power of the experiment if the direct procedure is used.

The reason the SID of Trp in corn and wheat middlings was less if calculated by the difference procedure than with the direct procedure may be that the SID of Trp in SBM was slightly overestimated because the SID of Trp in SBM was numerically greater than the SID for all other indispensable AA in SBM. Because of the low concentration of Trp in corn and wheat middlings, the majority of the Trp in the corn–SBM and the wheat middlings-SBM diets originated from SBM and a small overestimation of the SID of Trp in SBM may, therefore, have resulted in an underestimation of the SID of Trp in the test ingredients. The observation that Trp was the only indispensable AA in corn and wheat middlings, for which a reduced SID was observed for the difference procedure compared with the direct procedure, further indicates that this was a result of an overestimation of the SID of Trp in SBM. Whereas this overestimation was unintentional, these results demonstrate some of the challenges that may be associated with using the difference procedure.

Determination of SID values for AA in animal proteins and certain specialty proteins sometimes represent special challenges due to the special properties of these proteins in the small intestine of pigs, which sometimes results in very large endogenous secretions of AA into the small intestine. In these cases, it may be necessary to use the difference procedure to calculate SID values (Mateo and Stein, 2007; Almeida et al., 2013; Sulabo et al., 2013a, 2013b; Navarro et al., 2018). However, because results from this experiment demonstrate that SID values for all indispensable AA, except Trp, calculated using the difference procedure, are not different from values calculated using the direct procedure, the difference procedure can be used for animal and specialty proteins, but more replications may be needed to obtain a satisfactory power of the experiment.

Conclusion

The SID of indispensable AA in corn, wheat, and wheat middlings can be determined by either the direct or the difference procedure without influencing results. There is, therefore, no reason to be concerned about AA being included below the requirement as is often the case when the direct procedure is used to estimate SID of AA in cereal grains and grain co-products. For the ingredients used in this experiment, there was no advantage of using the difference procedure, but because of greater standard errors, more replicates are needed to obtain a certain power of the experiment if the difference procedure is used instead of the direct procedure.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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