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Non Ruminant Nutrition

NON RUMINANT NUTRITION

Crystalline amino acids do not influence calculated values for standardized ileal digestibility of amino acids in feed ingredients included in diets for pigs

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Abstract

An experiment was conducted to test the hypothesis that addition of crystalline amino acids (**AA**) to diets during the adaptation or collection periods will not influence calculated values for apparent ileal digestibility (**AID**) or standardized ileal digestibility (**SID**) of AA in corn and soybean meal (**SBM**). Seven ileal-cannulated barrows (initial body weight: 77.9 ± 2.6 kg) were allotted to a 7 × 7 Latin Square design with 7 dietary treatments and 7 periods. Dietary treatments included feeding diets based on corn or SBM without or with crystalline AA for the entire 7-d period or with crystalline AA during the adaptation period, but without crystalline AA during the collection period. An N-free diet was also used. Each experimental period consisted of 5 d of adaptation to the diets followed by 2 d of ileal digesta collection, with digesta being collected for 9 hr/d starting after feeding the morning meal. Thus, the entire experiment lasted 49 d. The AID and SID of crude protein (**CP**) and AA were calculated using values determined in corn or in SBM without or with crystalline AA to determine if crystalline AA influenced calculated values for AID or SID of CP and AA. Results indicated that addition of crystalline AA to diets fed during the entire 7-d period increased ($P < 0.05$) AID of some AA in corn and SBM. However, no differences in SID of CP and AA were observed between pigs fed the diets without crystalline AA and pigs fed the diets with crystalline AA if dietary crystalline AA were ignored in calculations of SID values. This indicates that crystalline AA were 100% absorbed before the distal ileum and did not affect calculated values for SID of AA if calculations were based only on the AA in corn or SBM. Therefore, it is concluded that crystalline AA may be added to experimental diets in digestibility experiments before and during collection periods without affecting results, if crystalline AA are disregarded in the calculation of AID or SID of AA in ingredients.

Key words: amino acid digestibility, crystalline amino acids, pigs, standardized ileal digestibility

Introduction

Standardized ileal digestibility (**SID**) of crude protein (**CP**) and amino acids (**AA**) needs to be determined to evaluate the quality of protein and AA in feed ingredients (Stein et al., 2007; NRC, 2012). The SID of CP and AA in feed ingredients is most commonly determined by the direct procedure, in which the experimental

Abbreviations

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

diet contains the test ingredient as the sole source of CP and AA. Therefore, the CP and AA supply from experimental diets do not always meet the requirement for SID AA by pigs, and as a consequence, pig growth and protein accretion in these pigs may be constrained. Protein status of pigs may affect endogenous secretions of AA, and therefore, values for AA digestibility may be underestimated due to the relatively greater contribution of endogenous AA and CP in the ileal digesta collected from pigs fed low-protein diets (Butts et al., 1993; Fan et al., 1994; Stein et al., 2005).

Experiments to determine the SID of CP and AA in feed ingredients typically utilize 7-d experimental periods, in which the initial 5 d of each period are used to adapt pigs to the diet, whereas ileal digesta are collected during the last 2 d of each period (Stein et al., 2007; Kim et al., 2020). If ingredients with low AA concentrations are used, a mixture of crystalline AA is sometimes added to the test diet during the adaptation phase to improve pig growth and reduce the severity of AA undernutrition (Pedersen et al., 2007; Casas et al., 2015). Crystalline AA are believed to be rapidly absorbed and 100% digestible (Chung and Baker, 1992) and addition of crystalline AA to diets during the adaptation phase is, therefore, believed not to influence SID of CP and AA during the collection phase, but this assumption has not been experimentally verified. However, if crystalline AA are indeed 100% digestible, it may be possible to include crystalline AA not only during the adaptation period but also in the diet consumed during the collection phase. This approach will theoretically not influence calculated values for SID of AA in the test feed ingredient if diet concentrations of crystalline AA are disregarded in the calculation of apparent ileal digestibility (**AID**) and SID of AA, but to our knowledge, this hypothesis has never been tested. Therefore, the objective of this experiment was to test the hypothesis that addition of crystalline AA to diets fed during the adaptation or collection periods will not influence calculated values for AID or SID of CP and AA in corn and soybean meal (**SBM**) if crystalline AA are disregarded in the calculations.

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 at Urbana-Champaign.

Animals and housing

Seven growing barrows that were the offspring of L359 boars mated to Camborough sows (Pig Improvement Company, Hendersonville, TN) were used. Pigs had an average body weight of 77.9 ± 2.6 kg at the start of the experiments and were surgically fitted with a T-cannula in the distal ileum for collection of ileal digesta (Stein et al., 1998). Pigs were housed in individual pens (1.2 × 1.5 m) in an environmentally controlled room, with lights turned on at all times. Pens had smooth, plastic-coated sides and fully slatted tribar metal floors. A top-fed individual dry feeder and a nipple drinker (AP AGCO, Paris, IL) were installed in each pen. All pigs had been used in a previous experiment and were fed a common corn–SBM diet for 14 d, before being allotted to the diets in this experiment. Pigs were allotted to a 7 × 7 Latin Square design with 7 treatments and seven 7-d periods (Kim and Stein, 2009). Therefore, there were a total of 7 observations per treatment.

Diets and feeding

The same batches of corn and SBM were used to prepare diets (Table 1). Two basal diets containing corn or SBM were formulated (Table 2). The diet containing SBM met AA requirements for 75 to 100 kg growing pigs (NRC, 2012), but the corn diet provided AA below the requirement. Two additional diets containing corn with crystalline AA or SBM with crystalline AA were also formulated. Addition of crystalline L-Lys HCl, DL-Met, L-Thr, L-Trp, L-His, L-Val, L-Ile, L-Phe, and L-Glu was necessary to meet the requirement for SID AA in the corn diet. The same quantities of crystalline AA were added to both diets resulting in the SBM diet containing AA above the requirement. The last diet was an N-free diet that was used to determine basal endogenous losses of AA and CP. Therefore, there were 7 experimental treatments: (1) the corn diet fed during the entire 7-d period; (2) the corn-crystalline AA diet fed during the initial 5 d (i.e., adaptation period), and the corn diet fed on days 6 and 7 (i.e., collection period); (3) the corn-crystalline AA diet fed during the entire 7-d period; (4) the SBM diet fed during the entire 7-d period; (5) the SBM-crystalline AA diet fed during adaption and the SBM diet fed during collection; (6) the SBM-crystalline AA diet fed during the entire 7-d period; and (7) N-free diet fed during the entire 7-d period (Figure 1). Chromic oxide was included in all diets at 0.40% as an indigestible marker, and vitamins and minerals were included to meet or exceed requirements for 75 to 100 kg growing pigs (NRC, 2012). Pigs were fed at a level of 3 times the

Table 1. Analyzed composition of corn and SBM (as-fed basis)

Item, %	Corn	SBM
Dry matter, %	87.01	89.79
Crude protein, %	6.63	47.61
Indispensable AA		
Arg	0.27	3.32
His	0.18	1.23
Ile	0.23	2.33
Leu	0.70	3.63
Lys	0.23	3.01
Met	0.12	0.67
Phe	0.29	2.47
Thr	0.23	1.78
Trp	0.05	0.72
Val	0.31	2.38
Dispensable AA		
Ala	0.44	2.00
Asp	0.41	5.36
Cys	0.15	0.71
Glu	1.07	8.47
Gly	0.27	2.10
Pro	0.54	2.54
Ser	0.28	1.93

Table 2. Ingredient composition of experimental diets (as-fed basis)[1]

Ingredient, %	Corn		SBM		
Crystalline AA	–	+	–	+	N-free diet
Ground corn	93.40	93.40	—	—	—
SBM, 48% crude protein	—	—	36.00	36.00	—
Ground limestone	1.00	1.00	0.80	0.80	0.45
Dicalcium phosphate	0.70	0.70	0.60	0.60	1.60
Sodium chloride	0.40	0.40	0.40	0.40	0.40
Chromic oxide	0.40	0.40	0.40	0.40	0.10
Soybean oil	—	—	3.0	3.0	4.00
Vitamin–mineral premix[2]	0.30	0.30	0.30	0.30	0.30
Corn starch	3.80	0.08	58.50	54.78	68.35
Sucrose	—	—	—	—	20.00
Potassium carbonate	—	—	—	—	0.40
Magnesium oxide	—	—	—	—	0.10
Solka floc	—	—	—	—	4.00
L-Lys HCl, 78% Lys	—	0.70	—	0.70	—
DL-Met	—	0.08	—	0.08	—
L-Thr	—	0.25	—	0.25	—
L-Trp	—	0.10	—	0.10	—
L-His	—	0.06	—	0.06	—
L-Val	—	0.18	—	0.18	—
L-Ile	—	0.17	—	0.17	—
L-Phe	—	0.18	—	0.18	—
Glu	—	2.00	—	2.00	—

[1]There were 3 treatments with each ingredient: 1 treatment without crystalline AA being fed for 7 d of each period; 1 treatment with the diet with crystalline AA being fed for 5 d and the diet without AA for the last 2 d of each period; and 1 treatment where the diet with crystalline AA was fed for all 7 d of each period.

[2]The vitamin–micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-α 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.

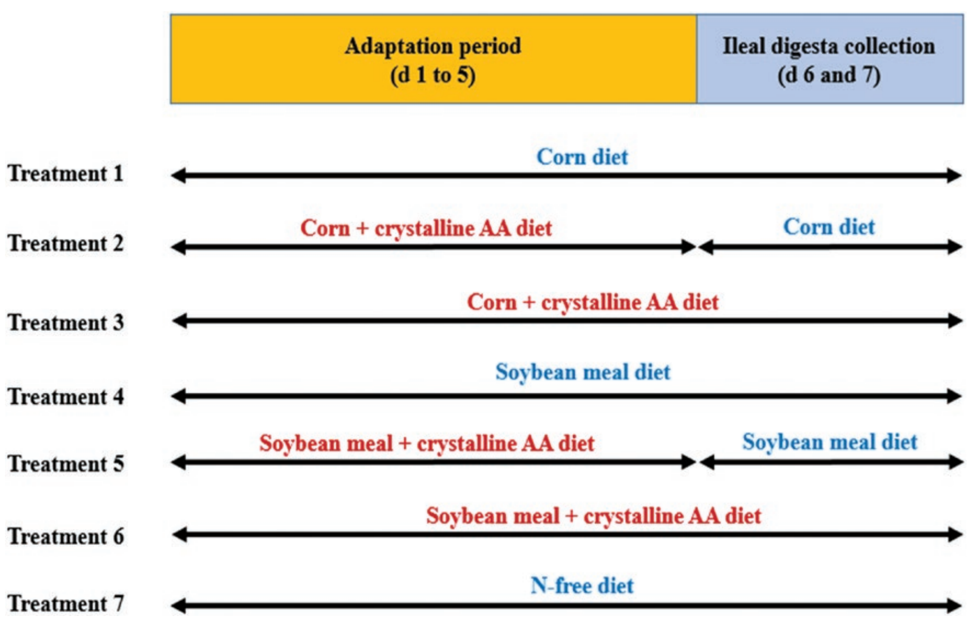

Figure 1. Dietary treatments in experiment included: (treatment 1) the corn diet was fed during the entire 7-d period; (treatment 2) the corn-crystalline AA diet was fed during the initial 5 d (i.e., adaptation period) and the corn diet was fed on days 6 and 7 (i.e., collection period); (treatment 3) the corn-crystalline AA diet was fed during the entire 7-d period; (treatment 4) the SBM diet was fed during the entire 7-d period; (treatment 5) the SBM-crystalline AA diet was fed during adaption and the SBM diet was fed during collection; (treatment 6) the SBM-crystalline AA diet was fed during the entire 7-d period; and (treatment 7) N-free diet was fed during the entire 7-d period.

Table 3. Analyzed chemical composition of experimental diets (as-fed basis)[1]

Item, %	Corn		SBM		
Crystalline AA	–	+	–	+	N-free diet
Dry matter	88.05	88.51	90.06	90.21	91.45
Crude protein	7.06	11.01	19.68	22.47	0.71
Indispensable AA					
Arg	0.30	0.30	1.25	1.19	0.02
His	0.18	0.26	0.47	0.51	0.01
Ile	0.22	0.37	0.85	1.00	0.02
Leu	0.70	0.71	1.37	1.30	0.04
Lys	0.23	0.77	1.13	1.57	0.02
Met	0.13	0.17	0.24	0.28	0.01
Phe	0.29	0.44	0.90	1.07	0.02
Thr	0.23	0.40	0.68	0.91	0.02
Trp	0.06	0.16	0.27	0.37	0.01
Val	0.31	0.51	0.88	1.02	0.03
Dispensable AA					
Ala	0.44	0.45	0.77	0.73	0.03
Asp	0.42	0.42	2.01	1.87	0.04
Cys	0.14	0.15	0.26	0.24	0.01
Glu	1.08	1.09	3.26	3.07	0.08
Gly	0.27	2.14	0.79	2.65	0.03
Pro	0.52	0.5	0.89	0.78	0.03
Ser	0.28	0.26	0.82	0.72	0.02

[1]There were 3 treatments with each ingredient: 1 treatment without crystalline AA being fed for 7 d of each period; 1 treatment with the diet with crystalline AA being fed for 5 d and the diet without AA for the last 2 d of each period; and 1 treatment where the diet with crystalline AA was fed for all 7 d of each period.

Table 4. AID (%) of CP and AA in corn without crystalline AA, with crystalline AA during the adaptation period (AA for 5 d), or with crystalline AA during the entire 7-d experimental period (AA for 7 d)[1, 2]

	Corn					
Item, %	No AA	AA for 5 d	AA for 7 d +[3]	AA for 7 d –[3]	SEM	P-values
CP	62.3[b]	64.6[b]	78.6[a]	67.8[ab]	3.92	0.028
Indispensable AA						
Arg	77.6	78.5	77.1	79.5	2.78	0.921
His	83.1	80.6	88.3	83.2	1.63	0.069
Ile	74.2[b]	72.2[b]	85.1[a]	75.5[b]	1.93	0.009
Leu	85.9	84.0	85.8	86.5	1.23	0.515
Lys	76.5[b]	75.8[b]	93.1[a]	76.1[b]	1.86	0.001
Met	86.4	84.0	88.8	86.6	1.18	0.170
Phe	80.3	76.7	86.4	80.2	1.90	0.072
Thr	62.9[b]	59.6[b]	80.0[a]	66.1[b]	3.49	0.026
Trp	56.2[b]	52.3[b]	85.9[a]	56.8[b]	3.59	0.001
Val	73.5[b]	71.5[b]	84.9[a]	75.1[b]	2.24	0.011
Mean	78.5[b]	76.5[b]	86.2[a]	79.4[b]	2.36	0.036
Dispensable AA						
Ala	76.9	76.8	77.9	78.8	1.88	0.922
Asp	71.5	70.4	70.5	72.7	2.26	0.920
Cys	76.5	72.9	75.6	76.1	1.94	0.605
Glu	83.6	82.1	82.6	83.7	1.80	0.833
Gly	35.3[b]	43.1[b]	94.1[a]	42.8[b]	7.66	0.001
Pro	–17.7	36.9	14.6	22.7	19.71	0.253
Ser	73.4	70.7	69.6	74.9	3.23	0.583
Mean	59.2	67.7	77.8	66.9	4.85	0.100
Total AA	67.9	71.7	81.7	72.7	3.58	0.077

[1]Data are least squares means of 7 observations for each treatment.
[2]There were 3 treatments with corn: 1 treatment without crystalline AA being fed for 7 d of each period; 1 treatment where the diet with crystalline AA was fed for 5 d and the diet without AA for the last 2 d of each period; and 1 treatment where the diet with crystalline AA was fed for all 7 d of each period.
[3]+ Calculations based on the corn diet with crystalline AA; – calculations based on the corn diet without crystalline AA.
[a–c]Values within a row lacking a common superscript letter are different ($P < 0.05$).

maintenance energy requirement (i.e., 197 kcal metabolizable energy per kilogram body weight$^{0.6}$; NRC, 2012).

Sample collection

Each period consisted of 5 d of adaptation to the diets followed by 2 d of ileal digesta collection, where collection was initiated after feeding the morning meal and ceased 9 hr later. For collection of samples, a 232-mL plastic bag was attached to the cannula barrel using a cable tie. Bags were removed when they were full or every 30 min and stored at −20 °C to prevent bacterial degradation of AA. At the conclusion of each period, digesta samples were thawed at room temperature and mixed within animal, and a subsample was collected. Digesta samples were lyophilized and finely ground prior to chemical analysis.

Chemical analyses

Ingredients, diets, and ileal digesta samples were analyzed in duplicate. After completing sample collections, ileal digesta samples were thawed, mixed within animal and diet, and a subsample was collected for chemical analysis. Digesta samples were lyophilized and finely ground before analysis. Diet, ingredient, and ileal digesta samples were analyzed for dry matter (Method 930.15; AOAC Int., 2007), and N was analyzed using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Aspartic acid was used as a calibration standard and CP was calculated as N × 6.25. AA were analyzed in diet, ingredient, and ileal digesta samples on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 hr at 110 °C (Method 982.30 E(a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (Method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 hr at 110 °C (Method 982.30 E(c); AOAC Int., 2007). The chromium concentration in diet and ileal digesta samples was determined using the Inductive Coupled Plasma Atomic Emission Spectrometric method (Method 990.08; AOAC Int., 2007). Samples were prepared using nitric acid–perchloric acid (Method 968.08 D(b); AOAC Int., 2007).

Calculations and statistical analyses

Following analysis, the AID and SID of CP and AA were calculated for each corn or SBM-containing diet (Stein et al., 2007). Values for AID and SID of AA in corn and SBM for the treatments that were fed no crystalline AA or crystalline AA only during the adaptation period were calculated based on the CP and AA concentration in the corn or SBM diets containing no crystalline AA. However, values for the treatments in which corn or SBM

Table 5. SID (%) of CP and AA in corn without crystalline AA, with crystalline AA during the adaptation period (AA for 5 d), or with crystalline AA during the entire 7-d experimental period (AA for 7 d)[1, 2, 3]

	Corn					
Item, %	No AA	AA for 5 d	AA for 7 d +[4]	AA for 7 d −[4]	SEM	*P*-values
CP	88.1	90.3	95.3	93.5	3.92	0.505
Indispensable AA						
Arg	97.1	98.1	98.1	99.1	2.78	0.963
His	91.4	88.9	93.6	91.5	1.63	0.347
Ile	85.8	83.8	91.7	87.1	1.93	0.140
Leu	91.2	89.2	91.3	91.8	1.23	0.502
Lys	88.6	88.0	96.2	88.3	1.86	0.081
Met	90.8	88.3	92.8	91.0	1.18	0.236
Phe	89.4	85.8	92.5	89.3	1.90	0.286
Thr	84.1	80.7	92.1	87.2	3.49	0.310
Trp	80.1	76.5	93.1	80.7	3.59	0.067
Val	84.7	82.8	91.5	86.3	2.24	0.128
Mean	89.4	87.5	93.3	90.3	2.36	0.311
Dispensable AA						
Ala	88.9	88.9	89.9	90.8	1.88	0.920
Asp	86.5	85.3	86.4	87.7	2.26	0.938
Cys	89.2	85.5	88.2	88.8	1.94	0.605
Glu	90.7	89.1	90.1	90.8	1.80	0.844
Gly	97.8	105.5	101.8	105.2	7.66	0.837
Pro	120.4	175.4	160.6	161.1	19.71	0.244
Ser	89.3	86.5	88.8	90.9	3.23	0.761
Mean	95.2	103.8	101.2	103.1	4.85	0.547
Total AA	92.6	96.4	97.8	97.4	3.58	0.698

[1]Data are least squares means of 7 observations for each treatment.
[2]SID values were calculated by correcting values for AID for the basal ileal endogenous losses. Basal ileal endogenous losses were determined as (g/kg of DM intake): CP, 20.64; Arg, 0.71; His, 0.16; Ile, 0.28; Leu, 0.44; Lys, 0.27; Met, 0.08; Phe, 0.30; Thr, 0.55; Trp, 0.13; Val, 0.38; Ala, 0.61; Asp, 0.75; Cys, 0.21; Glu, 0.92; Gly, 1.87; Pro, 8.25; and Ser, 0.56.
[3]There were 3 treatments with corn: 1 treatment without crystalline AA being fed for 7 d of each period; 1 treatment where the diet with crystalline AA was fed for 5 d and the diet without AA for the last 2 d of each period; and 1 treatment where the diet with crystalline AA was fed for all 7 d of each period.
[4]+ Calculations based on the corn diet with crystalline AA; − calculations based on the corn diet without crystalline AA.

diets with crystalline AA were fed during the entire 7-d period were calculated based on the diets containing crystalline AA and also based on the diets containing no crystalline AA, and thus, disregarding the presence of crystalline AA in the diets.

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The normality of residuals and outliers were 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. One pig fed the N-free diet in period 4 was identified as an outlier and removed from the data set. Data for AID and SID of CP and AA were first analyzed following a 2 × 3 factorial with 2 ingredients (i.e., corn or SBM) and 3 different calculations (i.e., no crystalline AA in the diet, crystalline AA in diet for 5 d, and crystalline AA in diet for 7 d). Pig and period were random effects. Because no interactions between ingredient and calculation were observed, data were subsequently analyzed using a model that included the ingredient as fixed effect and pig and period as random effects. The pig was the experimental unit for all analyses and differences were considered significant at $P < 0.05$, whereas $0.05 \le P < 0.10$ was considered a tendency.

Results

AA composition of ingredients

Analyzed concentrations of AA in corn and SBM indicated that corn contained 0.23% Lys, 0.12% Met, 0.23% Thr, and 0.05% Trp, whereas SBM contained 3.01% Lys, 0.67% Met, 1.78% Thr, and 0.72% Trp. Analyzed concentrations of DM, CP, and AA in experimental diets were in agreement with calculated values (Table 3).

AA digestibility in corn

The AID of CP, Ile, Lys, Thr, Trp, Val, Gly, and the mean of indispensable AA in corn was greater ($P < 0.05$) if crystalline AA were included in the diet during the entire 7-d period compared with AID values calculated for treatments without crystalline AA or with crystalline AA added only during the adaptation period (Table 4). The AID of His, Phe, and total AA also tended ($P < 0.10$) to be greater if crystalline AA were included in the diet during the entire 7-d period compared with the other corn treatments. However, if crystalline AA included in the diet were disregarded in the calculation, values for AID of CP and all AA in the corn diet were not different from AID values obtained in the corn diet with no crystalline AA or if crystalline AA were included in the diets only during the adaptation period.

Values for SID of CP and AA in corn were not influenced by addition of crystalline AA to the diets during the adaption period or during the entire 7-d period (Table 5). There was, however, a tendency for the SID of Lys and Thr to be greater ($P < 0.10$) if crystalline AA were added to the diet during the entire 7-d period. However, if inclusion of crystalline AA in the corn diet was ignored in the calculation of SID values, no differences among treatments were observed.

Table 6. AID (%) of CP and AA in SBM without crystalline AA, with crystalline AA during the adaptation period (AA for 5 d), or with crystalline AA during the entire 7-d experimental period (AA for 7 d)[1,2]

	SBM					
Item, %	No AA	AA for 5 d	AA for 7 d +[3]	AA for 7 d–[3]	SEM	P-values
CP	82.9	80.6	83.4	82.1	1.50	0.562
Indispensable AA						
Arg	92.1	91.1	90.1	91.1	0.76	0.124
His	90.8	88.7	89.7	89.5	0.83	0.428
Ile	87.7	86.1	88.3	87.0	0.83	0.300
Leu	88.2	86.6	85.4	86.9	0.91	0.223
Lys	92.6[ab]	91.2[b]	93.5[a]	91.5[b]	0.57	0.032
Met	89.5	88.7	89.4	88.2	0.61	0.315
Phe	87.6	86.2	88.2	86.7	0.92	0.425
Thr	82.5	80.3	84.7	80.8	1.32	0.067
Trp	84.6[bc]	82.3[c]	88.5[a]	85.1[b]	0.99	0.002
Val	85.4	83.4	85.7	84.4	1.14	0.408
Mean	88.7	87.0	88.5	87.5	0.84	0.440
Dispensable AA						
Ala	81.8	79.7	78.2	80.5	1.42	0.335
Asp	86.7	84.1	83.0	85.1	1.08	0.147
Cys	80.5	77.6	75.1	78.2	1.65	0.162
Glu	89.2	85.9	85.0	86.6	1.27	0.144
Gly	75.6[b]	69.3[b]	90.8[a]	70.8[b]	2.91	0.001
Pro	63.7	70.8	55.1	62.8	7.08	0.283
Ser	86.6[a]	84.8[a]	80.7[b]	83.9[ab]	1.28	0.020
Mean	83.7	80.4	82.8	81.4	1.84	0.593
Total AA	86.1	83.5	85.5	84.3	1.34	0.536

[1]Data are least squares means of 7 observations for each treatment.
[3]There were 3 treatments with corn: 1 treatment without crystalline AA being fed for 7 d of each period; 1 treatment where the diet with crystalline AA was fed for 5 d and the diet without AA for the last 2 d of each period; and 1 treatment where the diet with crystalline AA was fed for all 7 d of each period.
[4]+ Calculations based on the SBM diet with crystalline AA; – calculations based on the SBM diet without crystalline AA.
[a–c]Values within a row lacking a common superscript letter are different ($P < 0.05$).

AA digestibility in SBM

The AID of CP and most AA in SBM was not influenced by addition of crystalline AA to the diet during the adaption period or during the entire 7-d period (Table 6). However, the AID of Lys, Trp, Gly, and Ser was greater ($P < 0.05$) if crystalline AA were included in the diet during the entire 7-d period compared with treatments without crystalline AA in the collection period. The AID of Thr had a tendency to be greater ($P = 0.06$) if crystalline AA were included in the diet during the entire 7-d period compared with the other treatments. However, no differences among treatments were observed if crystalline AA were disregarded in the AID calculation.

The SID of Lys and Trp had a tendency ($P < 0.10$) to be greater in SBM if crystalline AA were included in the diets during the entire 7-d period compared with the other SBM treatments (Table 7). However, for the SID of CP and all other AA, inclusion of crystalline AA during the adaptation period or during the entire 7-d period had no influence on SID values. In addition, if the inclusion of crystalline AA in the collection period was disregarded in the calculation of SID values, no differences among treatments were observed.

Discussion

Crystalline AA mixtures are sometimes added to experimental diets during the adaptation period when conducting ileal digestibility experiments to meet AA requirements (Strang et al., 2016; Casas and Stein, 2017). The observation in this experiment that there was no effect on values for AID and SID of AA of adding crystalline AA to experimental diets during the adaptation period validates this praxis and confirms that crystalline AA fed during the adaptation period do not influence calculated values for AID or SID. The increase in AID of some AA that was observed in corn with added crystalline AA during the entire 7-d period compared with the AID of AA in corn without crystalline AA is likely a result of the greater AID of crystalline AA compared with the AID of AA in corn (Chung and Baker, 1992). In addition, contributions of endogenous AA to the total AA output in ileal digesta are greater if dietary AA concentrations are below the requirement compared with a diet in which AA concentrations are at the requirement (Donkoh and Moughan, 1994; Fan et al., 1994), which likely also contributed to the difference in AID of AA between the 2 treatments. The greater concentration of CP and AA in SBM compared with corn resulted in a lower contribution of crystalline AA to the AID and SID of CP and AA in SBM compared with corn, which is likely the reason AID and SID of CP and most AA in SBM were not affected by the addition of crystalline AA to SBM. The AID and SID of CP and AA were also greater in SBM than in corn, which partly negated the effect of adding crystalline AA with a greater digestibility. The impact of endogenous losses on AID of CP and AA is negligible if CP and AA exceeds a certain threshold level, which was suggested to be 15% CP and 0.94% Lys (Fan et al., 1994). The fact that CP and

Table 7. SID (%) of CP and AA in SBM fed without crystalline AA, with crystalline AA during the adaptation period (AA for 5 d), or with crystalline AA during the entire 7-d experimental period (AA for 7 d)[1,2,3]

	SBM					
Item, %	No AA	AA for 5 d	AA for 7 d +[4]	AA for 7 d –[4]	SEM	P-values
CP	92.4	90.0	91.7	91.5	1.50	0.720
Indispensable AA						
Arg	97.2	95.3	95.5	96.2	0.76	0.356
His	93.5	91.5	92.5	92.5	0.83	0.436
Ile	90.3	88.7	90.8	90.0	0.83	0.341
Leu	90.3	88.6	88.5	89.8	0.91	0.427
Lys	94.4	93.0	95.0	93.6	0.57	0.090
Met	91.5	90.1	91.9	91.1	0.61	0.255
Phe	90.1	88.6	90.7	89.7	0.92	0.457
Thr	88.5	86.1	90.1	88.0	1.32	0.182
Trp	90.3	88.2	91.7	89.5	0.99	0.068
Val	89.0	86.9	89.1	88.3	1.14	0.475
Mean	91.9	90.1	91.7	91.2	0.84	0.457
Dispensable AA						
Ala	88.0	85.8	85.7	87.6	1.42	0.557
Asp	89.2	86.5	86.7	88.5	1.08	0.271
Cys	86.6	83.6	83.1	85.6	1.65	0.411
Glu	91.0	87.5	87.7	89.2	1.27	0.237
Gly	96.1	89.6	97.2	92.2	2.91	0.276
Pro	145.8	139.6	150.5	146.4	7.08	0.844
Ser	90.6	88.6	87.8	90.2	1.28	0.385
Mean	96.2	92.6	94.6	94.9	1.84	0.619
Total AA	94.1	91.4	93.2	93.2	1.34	0.572

[1]Data are least squares means of 7 observations for each treatment.
[2]SID values were calculated by correcting values for AID for the basal ileal endogenous losses. Basal ileal endogenous losses were determined as (g/kg of DM intake): CP, 20.64; Arg, 0.71; His, 0.16; Ile, 0.28; Leu, 0.44; Lys, 0.27; Met, 0.08; Phe, 0.30; Thr, 0.55; Trp, 0.13; Val, 0.38; Ala, 0.61; Asp, 0.75; Cys, 0.21; Glu, 0.92; Gly, 1.87; Pro, 8.25; and Ser, 0.56.
[3]There were 3 treatments with corn: 1 treatment without crystalline AA being fed for 7 d of each period; 1 treatment where the diet with crystalline AA was fed for 5 d and the diet without AA for the last 2 d of each period; and 1 treatment where the diet with crystalline AA was fed for all 7 d of each period.
[4]+ Calculations based on the SBM diet with crystalline AA; – calculations based on the SBM diet without crystalline AA.

Lys and other AA exceeded the threshold levels in the diet based on SBM and no crystalline AA, is likely the reason that crystalline AA did not impact the AID of AA in SBM because there was no impact of endogenous losses on AID or SID of AA.

The reason values for AID and SID of CP and AA were calculated for the diets with crystalline AA as well as the diets without crystalline was to test the hypothesis that crystalline AA are 100% absorbed in the small intestine as has been suggested (Chung and Baker, 1992). If that is the case, crystalline AA were expected not to influence the concentration of AA in digesta collected at the distal ileum, and therefore, values for AID and SID were expected not to be affected by inclusion of crystalline AA in the diets. The observation that there were no differences in AID or SID among treatments if analyzed values for the corn or SBM diets without crystalline AA were used in the calculations demonstrates that crystalline AA are completely absorbed prior to the distal ileum, which is in agreement with the data by Chung and Baker (1992). The implication of this observation is that diets that are used in digestibility experiments that do not meet the requirements for AA may be supplemented with crystalline AA without affecting values for SID of AA if the crystalline AA are disregarded in the calculations. However, results also justify not to add crystalline AA to the diets because feeding diets in which AA are below the requirement do not change AID and SID of AA.

Addition of crystalline AA to corn resulted in a diet that was adequate in AA, which was not the case for the corn diet without AA. However, the SBM diet without AA contained AA in quantities that met the requirement, and as a consequence, addition of crystalline AA to the SBM diet resulted in a diet containing AA above the requirement. The observation that values for SID were not affected by inclusion of crystalline AA in the SBM diet, despite the fact that the diet contained AA well above the requirement, indicates that the capacity for AA absorption is not easily saturated in pigs. This observation is in agreement with data indicating that the AID of AA in SBM was not changed between diets containing 16.5 and 25.5% CP (Li et al., 1993). In addition, as dietary AA concentration increases from being at the requirement to above the requirement, the influence of changes in endogenous AA on AID of AA is negligible and values for AID plateaus at this point (Fan et al., 1994; Eklund et al., 2010). Within the range of dietary AA concentrations used in this experiment, it therefore appears that the efficiency of AA absorption was unaffected by dietary AA concentration.

Conclusion

Crystalline AA are 100% digestible and, therefore, may be added to experimental diets used in AA digestibility experiments to meet requirements of pigs without affecting calculated values for AID and SID in the diets if the crystalline AA are ignored in the calculations. Results also indicated that feeding pigs a diet that contains AA well above the requirement does not affect calculated values for AID and SID. In contrast, if ingredients with low AA concentrations are used, the AID and SID of AA are not influenced if these diets are fed without supplementation with crystalline AA although AA requirements are not being met.

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Conflict of interest statement

J.K.H. is an employee of Evonik Nutrition & Care GmbH, Hanau, Germany, which produces and markets crystalline AA, but all other authors have no conflicts of interest.

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