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Short communication

Concentrations of digestible amino acids in co-products from threonine and tryptophan fermentation are greater than in soybean meal

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ABSTRACT

An experiment was conducted to test the hypothesis that the standardized ileal digestibility of crude protein (CP) and amino acids (AA) in threonine biomass (Thr-BM) and 2 sources of tryptophan biomass (Trp-BM1 and Trp-BM2) were greater than in soybean meal (SBM). Twelve weanling barrows (11.08 ± 1.06 kg) that had a T-cannula installed in the distal ileum were allotted to a replicated 6 × 6 Latin square design with 6 diets and 6 periods. A nitrogen-free diet, a diet based on SBM, and a diet based on a combination of SBM and Thr-BM were formulated. Two additional diets were formulated based on a combination of SBM and the 2 sources of tryptophan biomass. The last diet was not related to this work. Diets were fed to pigs for 7 days, and ileal digesta were collected on days 6 and 7 of each period. Results indicated that concentrations of CP and AA were greater in biomass products than in SBM, and the AID of threonine was greater ($P < 0.01$) in the diet containing Thr-BM than in the SBM diet or the diets containing Trp-BM1 or Trp-BM2. The AID and SID of tryptophan were greater ($P < 0.01$) in diets containing Trp-BM1 or Trp-BM2 than in the SBM diet or Thr-BM diet. The AID and SID of aspartate were also greater ($P < 0.01$) in Trp-BM1 and Trp-BM2 than in Thr-BM. However, the AID and SID for all other AA in Thr-BM were not different from Trp-BM1 and Trp-BM2. The quantities of standardized ileal digestible CP and AA were also greater ($P < 0.01$) in biomass products compared with SBM. In conclusion, concentrations of standardardized ileal digestible CP and most AA in biomass products were greater than in SBM. Therefore, these biomass products may be used to replace other protein sources in diets fed to weanling pigs, but further research is needed to determine digestibility of energy and other nutrients, optimal inclusion rates, and effects of biomass products on pig growth performance and health.

1. Introduction

Soybean meal (SBM) is the most widely used plant source of protein in livestock and poultry diets due to its wide availability and

Abbreviations: AA, amino acids; AID, apparent ileal digestibility; CP, crude protein; EFSA, European Food Safety Authority; ND, not detected; SBM, soybean meal; SID, standardized ileal digestibility; Thr-BM, threonine biomass; Trp-BM1, tryptophan biomass 1; Trp-BM2, tryptophan biomass 2.

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excellent amino acid (AA) profile (Stein et al., 2008). However, SBM contains trypsin inhibitors, allergens, and non-digestible oligosaccharides (Grieshop et al., 2003), which may increase diarrhea incidence (Liying et al., 2003) and reduce growth performance (Hong et al., 2004) of weanling pigs. Therefore, it is necessary to evaluate ingredients that can be used as alternatives for plant and animal proteins in diets fed to weanling pigs.

Crystalline AA are often included in diets for pigs to meet requirements for indispensable AA. Reducing the concentration of crude protein (CP) by 20–40 g/kg with increased inclusion of crystalline AA in pig diets may result in increased utilization of nitrogen, reduction in post weaning diarrhea, and increased growth performance of pigs (Stein and Kil, 2006; Kil and Stein, 2010; Wang et al., 2018). Therefore, the availability of crystalline AA has increased to provide AA required by pigs to maximize growth and improve intestinal health. Some of the crystalline AA produced include crystalline L-threonine and L-tryptophan, and these AA are commercially produced via large-scale fermentation technologies (Krämer, 2004). Crystalline L-threonine and L-tryptophan are produced via direct fermentation using strains of *Escherichia coli*, *Serratia marcescens*, *Brevibacterium flavum*, and *Corynebacterium glutamicum,* which are efficient in synthesizing specific AA. These specific strains are incubated in a culture medium using carbohydrates and nitrogen as substrates (Chen et al., 2009). After the fermentation process, crystalline L-threonine and L-tryptophan are harvested from the biomass, but the remaining biomass is rich in CP, AA, and phosphorus. Indeed, the fermentation biomass from crystalline L-Thr production had greater concentrations of digestible Thr and metabolizable energy than fish meal (Almeida et al., 2014).

These new sources of biomass products can possibly be used as protein sources in diets fed to pigs, but information about the nutritional value of the microbial biomass is limited. Therefore, the objective of this work was to determine the apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of CP and AA in threonine and tryptophan biomass fed to weanling pigs, and to test the hypothesis that the concentration of standardized ileal digestible CP and AA in biomass products were greater than in SBM.

2. 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, USA (approval number 15196). Pigs used in the experiment were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

2.1. Ingredients, diets, animals, housing, and experimental design

A source of threonine biomass (Thr-BM), 2 sources of tryptophan biomass (Trp-BM1 and Trp-BM2), and SBM were used (Tables 1 and 2). The Thr-BM was derived from a non-pathogenic *Escherichia coli* K12 (DSM 25086), which is a safe production strain that has

Table 1
Analyzed chemical composition of ingredients.

Item, g/kg	Soybean meal	Thr-BM[1]	Trp-BM1[1]	Trp-BM2[1]
Dry matter	894.4	942.9	951.5	951.3
Gross energy, MJ/kg	17.4	21.7	22.6	22.8
Crude protein	441.1	811.6	709.7	725.3
Non-protein nitrogen	–	13.5	15.1	12.7
Soluble dietary fiber	25.0	13.0	ND[2]	ND
Insoluble dietary fiber	158.0	16.0	73.0	50.0
Total dietary fiber	183.0	29.0	73.0	50.0
AEE[2]	10.0	53.2	102.0	116.4
Ash	59.6	32.4	67.2	70.4
Calcium	4.2	0.3	0.2	0.3
Phosphorus	5.7	5.2	7.2	6.9
Indispensable amino acids				
Arginine	32.4	48.1	38.4	39.4
Histidine	11.3	17.2	15.5	15.8
Isoleucine	19.8	38.4	32.1	32.9
Leucine	33.2	66.6	56.5	57.2
Lysine	27.2	47.2	40.5	41.0
Methionine	6.0	20.8	16.9	17.2
Phenylalanine	21.4	32.0	28.1	28.5
Threonine	17.0	120.1	29.0	29.2
Tryptophan	6.1	10.0	76.8	68.9
Valine	20.9	46.0	37.2	38.0
Dispensable amino acids				
Alanine	18.6	54.5	45.0	45.6
Aspartate	48.9	77.5	66.1	66.8
Cysteine	6.5	5.6	5.8	5.8
Glutamate	79.4	93.6	78.8	79.6
Glycine	18.6	36.3	30.9	31.3
Serine	22.1	28.9	24.0	24.5

[1]Thr-BM, threonine biomass; Trp-BM1, tryptophan biomass 1; Trp-BM2, tryptophan biomass 2; Evonik Operations GmbH, Hanau, Germany.
[2]ND, not detected; AEE, acid hydrolyzed ether extract.

been widely utilized in microbial fermentation. The 2 sources of tryptophan biomass were produced by *Escherichia coli* strain DSM 25084; they were produced at the same fermentation unit and based on the same substrates, but the 2 sources originated from 2 different batches. The Thr-BM, Trp-BM1, and Trp-BM2 were produced and supplied by a commercial company (Evonik Operations GmbH, Hanau, Germany).

One diet was based on SBM, one diet was based on a combination of SBM and Thr-BM, and 2 diets were based on a combination of SBM and Trp-BM1 or Trp-BM2. A nitrogen-free diet that was used to determine basal endogenous losses of CP and AA was also formulated. Thus, 5 diets were used (Table 3). An additional diet that was unrelated to this work was also included in the animal trial. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). All diets also contained 4 g/kg chromic oxide as an indigestible marker.

Twelve weanling barrows (initial body weight: 11.08 ± 1.06 kg) were surgically fitted with a T-cannula in the distal ileum (Stein et al., 1998), and allotted to a replicated 6 × 6 Latin square design with 6 diets and 6 periods in each square (Kim and Stein, 2009). There was, therefore, a total of 12 observations per treatment. Pigs were placed in individual pens (1.2 × 1.5 m) that were equipped with a self-feeder, a nipple waterer, and a slatted tri-bar floor.

All pigs were fed at 3.0 times the maintenance energy requirement (i.e., 0.824 MJ kg body weight$^{0.60}$; NRC, 2012) and water was available at all times throughout the experiment. Pig weights were recorded at the beginning and at the end of each period and the amount of feed supplied each day was recorded. Pigs were allowed to recover from surgery for 7 days prior to being fed experimental diets. The initial 5 days of each period was considered an adaptation period to the diet. Ileal digesta were collected for 8 h on days 6 and 7 as described by Stein et al. (1998). On the completion of one experimental period, animals were deprived of feed overnight and the following morning, a new experimental diet was offered.

2.2. Chemical analyses

Ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was collected for chemical analysis. Digesta samples were lyophilized and finely ground in a coffee grinder prior to chemical analysis. Ingredient, diet, and ileal digesta samples were analyzed for dry matter (Method 930.15; AOAC Int., 2007), and N was analyzed N was 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, USA). Crude protein was calculated as N × 6.25. Ingredients were analyzed for ash (Method 942.05; AOAC Int., 2007) and for acid hydrolyzed ether extract by acid hydrolysis using 3*N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA)

Table 2
Concentrations of phospholipid, free fatty acid, and fatty acid profile of threonine biomass (Thr-BM), tryptophan biomass 1 (Trp-BM1), and tryptophan biomass 2 (Trp-BM2).

Item	Thr-BM	Trp-BM1	Trp-BM2
Phospholipid, g/kg of total fat	33.5	92.5	91.6
Free fatty acids, g/kg of total fat	630.0	403.3	371.8
Fatty acid profile, g/kg of total fat[1]			
C6:0	0.5	0.6	0.5
C8:0	0.8	1.0	0.9
C10:0	4.9	1.2	1.2
C11:0	2.6	0.7	1.0
C12:0	24.8	42.8	38.9
C13:0	3.7	4.8	6.2
C14:0	100.0	101.9	96.3
C14:1	0.1	0.2	0.2
C15:0	36.1	39.4	54.3
C15:1	0.1	1.5	1.8
C16:0	455.6	378.6	376.6
C16:1	6.3	144.4	143.0
C17:0	251.7	125.3	131.6
C17:1	0.9	2.0	2.6
C18:0	6.6	7.0	7.2
C18:1	7.1	73.8	70.6
C18:2	0.5	12.0	0.4
C19:0	69.0	12.1	13.5
C20:1	0.4	0.3	0.1
C20:5	ND[2]	9.1	3.8
Total saturated fatty acids[3]	956.3	715.4	728.2
Total monounsaturated fatty acids[4]	14.9	222.2	218.3
Total polyunsaturated fatty acids[5]	0.5	21.1	4.2

[1]C18:3, C20:0, C20:2, C20:3, C20:4, C20:5, C21:0, C22:0, C22:1, C22:6, C23:0, C24:0, and C24:1 were analyzed, but concentrations were below detectable levels in all samples.
[2]ND, not detected.
[3]Total saturated fatty acids, C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0.
[4]Total monounsaturated fatty acids, C14:1 + C15:1 + C16:1 + C17:1 + C18:1 + C20:1.
[5]Total polyunsaturated fatty acids, C18:2 + C20:5.

Table 3
Composition of experimental diets (as-fed basis).

Ingredient, g/kg	Soybean meal	Thr-BM[1]	Trp-BM1[1]	Thr-BM2[1]	Nitrogen-free
Soybean meal	420.0	150.0	150.0	150.0	–
Thr-BM	–	160.0	–	–	–
Trp-BM1	–	–	180.0	–	–
Trp-BM2	–	–	–	180.0	–
Soybean oil	30.0	30.0	30.0	30.0	40.0
Solka floc	10.0	20.0	20.0	20.0	30.0
Dicalcium phosphate	13.0	15.0	15.0	15.0	20.0
Limestone	10.0	10.0	10.0	10.0	8.0
Sugar	150.0	150.0	150.0	150.0	200.0
Chromic oxide	4.0	4.0	4.0	4.0	4.0
Cornstarch	356.0	454.0	434.0	434.0	686.0
Magnesium oxide	–	–	–	–	1.0
Potassium carbonate	–	–	–	–	4.0
Salt	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix[2]	3.0	3.0	3.0	3.0	3.0
Analyzed composition, g/kg					
Dry matter	911.2	918.4	921.1	922.7	918.8
Crude protein	212.3	229.3	210.2	207.8	12.6
Indispensable amino acids					
Arginine	14.4	13.9	12.0	12.2	0.07
Histidine	5.0	4.9	4.5	4.6	0.03
Isoleucine	9.1	10.2	8.9	9.2	0.07
Leucine	15.1	17.7	15.6	15.9	0.20
Lysine	12.2	13.0	11.5	11.8	0.07
Methionine	2.7	4.6	4.0	4.0	0.03
Phenylalanine	9.8	9.3	8.5	8.7	0.06
Threonine	7.6	24.7	7.9	8.0	0.05
Tryptophan	2.9	2.8	14.2	13.0	0.03
Valine	9.5	11.8	10.0	10.3	0.12
Dispensable amino acids					
Alanine	8.4	13.2	11.1	11.3	0.14
Aspartate	22.3	22.3	19.6	20.0	0.15
Cysteine	3.0	2.1	2.1	2.1	0.07
Glutamate	36.0	30.0	26.8	27.4	0.32
Glycine	8.4	9.7	8.6	8.7	0.09
Serine	9.9	9.0	7.9	7.9	0.08

[1]Thr-BM, threonine biomass; Trp-BM1, tryptophan biomass 1; Trp-BM2, tryptophan biomass 2; Evonik Operations GmbH, Hanau, Germany.
[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 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 B12, 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.

followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA). Ingredients were analyzed for gross energy using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Ingredients were also analyzed for insoluble dietary fiber and soluble dietary fiber according to method 991.43 (AOAC Int., 2007) using the Ankom TDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). Total dietary fiber was calculated as the sum of insoluble dietary fiber and soluble dietary fiber. Calcium and phosphorus were analyzed in ingredients using inductively coupled plasma optical emissions spectrometry using an internally validated method (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Amino acids were analyzed in ingredient, diet, and ileal digesta samples by ion-exchange chromatography using ninhydrin for post-column derivatization. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110 °C and quantified with the internal standard by measuring the absorption of reaction products with ninhydrin. Methionine and cysteine were determined by cold performic acid oxidation overnight, and were neutralized with Na metabisulfite (Llames and Fontaine, 1994). Tryptophan was determined by high-performance liquid chromatography after NaOH hydrolysis with barium hydroxide octahydrate for 20 h at 110 °C (Almeida et al., 2013). The biomass products were analyzed for non-protein nitrogen following the procedure of Prigge et al. (1976). Concentrations of phospholipids were analyzed in the biomass products using the Folch extraction procedure (Folch et al., 1957). Methyl esters of fatty acids were extracted from the 3 biomass products (Method Ce-266; AOCS, 2017), and the concentrations of fatty acids in the 3 biomass products were measured using a capillary gas liquid chromatography (Method 996.06; AOAC Int., 2007). The chromium concentration was analyzed in diet and ileal digesta samples (Method 990.08; AOAC Int., 2007). Samples for chromium analysis were prepared using nitric acid-perchloric acid [Method 968.08 D(b); AOAC Int., 2007].

Table 4
Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) coefficients of crude protein (CP) and amino acids (AA) by weanling pigs fed experimental diets[1].

	AID						SID[2]					
Item	SBM[3]	Thr-BM[3]	Trp-BM1[3]	Trp-BM2[3]	SEM	P-value	SBM	Thr-BM	Trp-BM1	Trp-BM2	SEM	P-value
CP	0.832[a]	0.776[b]	0.764[b]	0.773[b]	0.0191	0.035	0.928[a]	0.866[b]	0.863[b]	0.873[b]	0.0191	0.034
Indispensable AA												
Arginine	0.922[a]	0.879[ab]	0.869[b]	0.886[ab]	0.0157	0.042	0.968	0.928	0.926	0.942	0.0157	0.102
Histidine	0.882[a]	0.831[b]	0.824[b]	0.840[b]	0.0157	0.014	0.925[a]	0.875[b]	0.872[b]	0.887[b]	0.0157	0.024
Isoleucine	0.865	0.842	0.818	0.834	0.0148	0.098	0.902	0.876	0.856	0.871	0.0148	0.110
Leucine	0.865	0.847	0.824	0.837	0.0145	0.151	0.904	0.880	0.862	0.874	0.0145	0.144
Lysine	0.882	0.845	0.843	0.862	0.0154	0.133	0.914	0.875	0.877	0.895	0.0154	0.133
Methionine	0.882	0.850	0.860	0.867	0.0107	0.126	0.919[a]	0.872[b]	0.886[b]	0.892[ab]	0.0107	0.011
Phenylalanine	0.852[a]	0.805[b]	0.780[b]	0.796[b]	0.0195	0.025	0.899[a]	0.855[b]	0.836[b]	0.850[b]	0.0195	0.050
Threonine	0.793[b]	0.901[a]	0.719[c]	0.730[c]	0.0211	<0.001	0.892[a]	0.932[a]	0.816[b]	0.827[b]	0.0211	<0.001
Tryptophan	0.851[b]	0.761[c]	0.940[a]	0.939[a]	0.0098	<0.001	0.913[b]	0.824[c]	0.953[a]	0.953[a]	0.0098	<0.001
Valine	0.844	0.818	0.791	0.807	0.0161	0.079	0.895	0.858	0.839	0.854	0.0161	0.060
Total	0.868	0.853	0.834	0.845	0.0142	0.263	0.915	0.890	0.878	0.889	0.0142	0.180
Dispensable AA												
Alanine	0.807	0.786	0.787	0.800	0.0163	0.663	0.884	0.836	0.846	0.858	0.0163	0.113
Aspartate	0.847[a]	0.697[c]	0.781[b]	0.806[b]	0.0158	<0.001	0.887[a]	0.737[c]	0.827[b]	0.852[ab]	0.0158	<0.001
Cysteine	0.777[a]	0.592[b]	0.500[c]	0.549[bc]	0.0432	<0.001	0.880[a]	0.739[b]	0.651[b]	0.701[b]	0.0431	<0.001
Glutamate	0.877[a]	0.798[b]	0.808[b]	0.840[ab]	0.0207	0.009	0.905[a]	0.832[b]	0.846[b]	0.877[ab]	0.0207	0.018
Glycine	0.711	0.637	0.582	0.585	0.0401	0.056	0.969[a]	0.861[b]	0.834[b]	0.836[b]	0.0401	0.041
Serine	0.838[a]	0.765[b]	0.726[b]	0.746[b]	0.0211	0.001	0.909[a]	0.844[b]	0.817[b]	0.837[b]	0.0211	0.008
Total	0.809[a]	0.708[b]	0.739[b]	0.766[ab]	0.0261	0.024	0.933[a]	0.837[b]	0.886[ab]	0.910[a]	0.0261	0.032
All AA	0.837	0.787	0.790	0.806	0.0194	0.138	0.925	0.866	0.881	0.899	0.0194	0.091

[a-c]Values within a row lacking a common superscript letter differ ($P < 0.05$).
[1]Data are least squares means of 9, 10, or 11 observations for each treatment.
[2]Standardized ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Basal ileal endogenous losses were determined as (g/kg of DMI): CP, 22.49; Arg, 0.74; His, 0.24; Ile, 0.37; Leu, 0.64; Lys, 0.42; Met, 0.11; Phe, 0.51; Thr, 0.84; Trp, 0.19; Val, 0.52; Ala, 0.71; Asp, 0.99; Cys, 0.34; Glu, 1.12; Gly, 2.36; and Ser, 0.78.
[2]SBM, soybean meal; Thr- BM, threonine biomass; Trp-BM1, tryptophan biomass 1; Trp-BM2, tryptophan biomass 2; Biomass products were provided by Evonik Operations GmbH, Hanau, Germany.

2.3. Calculations and statistical analyses

Values for AID, ileal endogenous losses, and SID of CP and AA in each diet were calculated according to Stein et al. (2007). The AID and SID for CP and AA in the SBM diet also represented the AID and SID of CP and AA of SBM because SBM was the only source of CP and AA in this diet. However, for the other diets, AID and SID values represented the combination of the AID and SID of CP and AA from SBM and Thr-BM, Trp-BM1, or Trp-BM2, and the AID and SID of CP and AA in Thr-BM, Trp-BM1, and Trp-BM2 were, therefore, calculated using the difference procedure (Fan and Sauer, 1995). Concentrations of standardized ileal digestible CP and AA were calculated by multiplying the SID coefficient by the CP and AA content in Thr-BM, Trp-BM1, Trp-BM2, and SBM.

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., 2016). Homogeneity of variances was confirmed using the UNIVARIATE procedure and outliers were tested using the BOXPLOT procedure and outliers were detected as observations that deviated from the treatment mean by ± 3 times the interquartile range. The model included diet or ingredient as the fixed effect and pig and period as random effects. Least squares means were calculated for each independent variable. Means were separated using the PDIFF option of SAS. The pig was the experimental unit and significance among means was assessed with an α level of 0.05.

3. Results

Concentrations of CP and AA were greater in biomass products than in SBM. The AID of CP and some AA were greater ($P < 0.05$) in the SBM diet than in diets containing Thr-BM, Trp-BM1, or Trp-BM2 (Table 4). The SID of histidine and phenylalanine was also greater ($P < 0.05$) in the SBM diet than in other diets, and the SID of methionine was greater ($P < 0.05$) in the SBM diet than in diets containing Thr-BM or Trp-BM1. The AID of threonine was greater ($P < 0.01$) in the diet containing Thr-BM than in the SBM diet, and the AID and SID of threonine were greater ($P < 0.01$) than in diets containing Trp-BM1 or Trp-BM2. The AID and SID of tryptophan were greater ($P < 0.01$) in diets containing Trp-BM1 or Trp-BM2 than in the SBM diet or the Thr-BM diet. The AID and SID of tryptophan in the SBM diet were also greater ($P < 0.01$) than in the Thr-BM diet.

The AID and SID of tryptophan and aspartate were greater ($P < 0.01$) in Trp-BM1 and Trp-BM2 than in Thr-BM, whereas the AID and SID of threonine were greater ($P < 0.01$) in Thr-BM than in 2 sources of tryptophan biomass (Table 5). The AID and SID of all other AA in Thr-BM were not different from values calculated for Trp-BM1 and Trp-BM2. CConcentrations of standardized ileal digestible CP, all indispensable AA except tryptophan, alanine, and glycine were greatest ($P < 0.01$) in Thr-BM, followed by the tryptophan biomass products, and SBM (Table 6). Concentrations of standardized ileal digestible cysteine and serine were greatest ($P < 0.01$) in SBM and in Thr-BM, respectively. The tryptophan biomass products had the greatest ($P < 0.01$) concentration of standardized ileal digestible tryptophan and aspartate, followed by Thr-BM, and SBM.

Table 5
Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) coefficients of crude protein (CP) and amino acids (AA) in threonine biomass (Thr-BM) and in 2 sources of tryptophan biomass (Trp-BM1 and Trp-BM2)[1].

Item	AID					SID				
	Thr-BM[2]	Trp-BM1[2]	Trp-BM2[2]	SEM	P-value	Thr-BM	Trp-BM1	Trp-BM2	SEM	P-value
CP	0.749	0.733	0.740	0.0308	0.877	0.836	0.832	0.841	0.0308	0.967
Indispensable AA										
Arginine	0.871	0.834	0.859	0.0310	0.508	0.922	0.898	0.921	0.0310	0.691
Histidine	0.800	0.789	0.809	0.0277	0.759	0.845	0.840	0.859	0.0277	0.792
Isoleucine	0.830	0.793	0.814	0.0242	0.370	0.862	0.832	0.852	0.0242	0.511
Leucine	0.838	0.804	0.820	0.0233	0.419	0.868	0.841	0.856	0.0233	0.563
Lysine	0.824	0.821	0.845	0.0257	0.676	0.853	0.857	0.879	0.0257	0.627
Methionine	0.841	0.854	0.862	0.0140	0.470	0.859	0.876	0.884	0.0140	0.340
Phenylalanine	0.774	0.734	0.756	0.0353	0.533	0.826	0.794	0.814	0.0353	0.664
Threonine	0.914[a]	0.682[b]	0.694[b]	0.0340	<0.001	0.936[a]	0.779[b]	0.789[b]	0.0340	0.002
Tryptophan	0.709[b]	0.946[a]	0.945[a]	0.0143	<0.001	0.773[b]	0.956[a]	0.955[a]	0.0143	<0.001
Valine	0.806	0.766	0.786	0.0257	0.354	0.842	0.813	0.831	0.0257	0.552
Total	0.846	0.819	0.832	0.0219	0.522	0.879	0.861	0.873	0.0219	0.731
Dispensable AA										
Alanine	0.778	0.780	0.794	0.0218	0.816	0.819	0.833	0.846	0.0218	0.620
Aspartate	0.607[b]	0.740[a]	0.777[a]	0.0273	0.001	0.648[b]	0.790[a]	0.825[a]	0.0273	<0.001
Cysteine	0.394	0.244	0.318	0.0933	0.190	0.590	0.441	0.516	0.0933	0.192
Glutamate	0.735	0.752	0.799	0.0398	0.312	0.774	0.799	0.844	0.0398	0.261
Glycine	0.598	0.521	0.512	0.0605	0.453	0.806	0.770	0.760	0.0605	0.803
Serine	0.712	0.641	0.670	0.0409	0.243	0.797	0.747	0.775	0.0409	0.476
Total	0.643	0.695	0.732	0.0446	0.293	0.775	0.856	0.889	0.0446	0.135
All AA	0.760	0.766	0.789	0.0309	0.726	0.835	0.859	0.880	0.0308	0.487

[a-c]Values within a row lacking a common superscript letter differ ($P < 0.05$).
[1]Data are least squares means of 9, 10, or 11 observations for each treatment.
[2]Evonik Operations GmbH, Hanau, Germany.

Table 6
Concentrations (g/kg) of standardized ileal digestible crude protein (CP) and amino acids (AA) in soybean meal (SBM), threonine biomass (Thr-BM), and in 2 sources of tryptophan biomass (Trp-BM1 and Trp-BM2)[1].

Item	SBM	Thr-BM[2]	Trp-BM1[2]	Thr-BM2[2]	SEM	P-value
Crude protein	409.3[c]	679.2[a]	590.8[b]	613.4[b]	20.05	<0.001
Indispensable amino acids						
Arginine	31.4[c]	44.1[a]	34.5[b]	36.4[b]	1.03	<0.001
Histidine	10.4[c]	14.5[a]	13.0[b]	13.7[ab]	0.36	<0.001
Isoleucine	17.9[c]	33.1[a]	26.7[b]	28.1[b]	0.66	<0.001
Leucine	30.0[c]	57.8[a]	47.6[b]	49.1[b]	1.17	<0.001
Lysine	24.8[c]	40.3[a]	34.7[b]	36.2[b]	0.93	<0.001
Methionine	5.5[c]	17.8[a]	14.8[b]	15.2[b]	0.21	<0.001
Phenylalanine	19.3[c]	26.4[a]	22.4[b]	23.3[b]	0.88	<0.001
Threonine	15.2[c]	112.5[a]	22.6[b]	23.1[b]	0.92	<0.001
Tryptophan	5.6[d]	7.7[c]	73.4[a]	65.9[b]	0.32	<0.001
Valine	18.7[c]	38.8[a]	30.2[b]	31.7[b]	0.85	<0.001
Total	178.8[c]	392.4[a]	319.6[b]	322.3[b]	7.25	<0.001
Dispensable amino acids						
Alanine	16.5[c]	44.7[a]	37.5[b]	38.6[b]	0.85	<0.001
Aspartate	43.5[c]	50.2[b]	52.2[ab]	55.4[a]	1.61	<0.001
Cysteine	5.7[a]	3.3[b]	2.6[b]	3.1[b]	0.48	<0.001
Glutamate	72.0[a]	72.5[a]	63.0[b]	67.9[ab]	2.94	0.022
Glycine	18.1[c]	29.3[a]	23.8[b]	24.0[b]	1.75	<0.001
Serine	20.1[b]	23.1[a]	17.9[c]	19.1[bc]	0.87	<0.001
Total	201.9[b]	251.7[a]	234.9[a]	248.4[a]	11.48	0.009
All AA	380.8[c]	644.1[a]	554.6[b]	571.1[b]	18.27	<0.001

[a-c]Values within a row lacking a common superscript letter differ ($P < 0.05$).
[1]Concentrations of standardized ileal digestible CP and AA were calculated by multiplying values for the standardized ileal digestibility coefficient of CP and AA by the concentration of CP and AA in each feed ingredient.
[2]Evonik Operations GmbH, Hanau, Germany.

4. Discussion

The safety of crystalline AA, including that of Thr produced from strain DSM 25086, has been assessed by the European Food Safety Authority (EFSA) and it was concluded that neither the production strain nor its recombinant DNA was present in the final Thr product (EFSA FEEDAP, 2014). Production of biomasses from genetically modified organisms such as DSM 25086 follow principles outlined in relevant guidance documents (EFSA, 2011a, 2011b). Likewise, production of biomass from AA produced by non-genetically modified organisms such as DSM 25084, which was used in the production of the Trp biomass, follows established guidelines (EFSA, 2011a, 2015; 2017).

The observation that the concentration of non-protein nitrogen was very low demonstrates that the AA containing protein makes up almost all the protein in the biomasses used in this experiment, which has also been reported for a different source of Thr biomass (Almeida et al., 2014). The fact that most of the fat was saturated and that most of the fat consisted of C16:0 and C17:0 indicates that the fat may have been of microbial origin. This fatty acid composition is different from the fatty acid composition in most plant feed ingredients (NRC, 2012).

A basal diet based on SBM was included in this experiment and the difference procedure was used to determine digestibility of AA in Thr-BM, Trp-BM1, and Trp-BM2. A consequence of using the difference procedure is that reliable results for the test ingredients will be obtained only if the digestibility coefficients of AA in the ingredient included in the basal diet are accurate (Oliveira et al., 2020). The AID and SID of AA in SBM used in the experiment are in close agreement with previous data (Cervantes-Pahm and Stein, 2010; NRC, 2012), which gives confidence that calculated coefficients for the AID and SID in Thr-BM, Trp-BM1, and Trp-BM2 are also accurate.

The observation that the concentration of digestible threonine was greater in Thr-BM than in Trp-BM1 and Trp-BM2, and that the concentration of digestible tryptophan was greater in the 2 sources of tryptophan biomass than in Thr-BM, indicates that the removal of crystalline AA from the biomass is not a completely efficient process, and that residual quantities of the AA that was produced remains in the biomass. These observations are in agreement with data indicating that the concentration of digestible lysine is greater in fermentation biomass produced from L-lysine production than in fish meal and hydrolyzed porcine intestinal mucosa products (Sulabo et al., 2013). Likewise, the concentration of valine is also elevated in a valine biomass (Oliveira et al., 2019), and the concentration of digestible threonine is greater in a different source of Thr-BM compared with fish meal (Almeida et al., 2014). Thus, it appears that the biomass left after production of crystalline AA may be used to supply the AA that was produced during fermentation. The observation that the digestibility of some AA in Thr-BM, Trp-BM1, or Trp-BM2 diets is less than in the SBM diet is in contrast with data indicating that values for AA digestibility in lysine biomass are not different from that of SBM (Sulabo et al., 2013). Values for AID and SID of most AA in Thr-BM used in this experiment are also less than previously reported values (Almeida et al., 2014). These observations may be attributed to differences in the fermentation process used for L-tryptophan and L-threonine production. However, it is also possible that the source of nitrogen and the fermentation conditions may influence quantity and digestibility of AA in the biomass. Nevertheless, the relatively high concentration of protein and high digestibility of AA resulted in a greater concentration of standardized ileal digestible AA being provided per unit of weight by the biomass products than by SBM used in this experiment. A similar observation

was reported by Sulabo et al. (2013) and indicates that the biomass that is left after crystalline AA production is a high quality feed ingredient that is well digested by pigs.

5. Conclusion

The threonine and tryptophan biomass products used in the present experiment were rich sources of crude protein and amino acids, and contained greater quantities of standardized ileal digestible amino acids than soybean meal. Therefore, these biomass products may be used to replace other protein sources in diets fed to weanling pigs, but further research is warranted to determine optimal inclusion rate of these biomass products. There is also a need to determine digestibility of energy and nutrients other than amino acids in the biomass products before they can be included in diets for pigs.

Author statement

JKH and HHS conceptualized the experiment, CDE analyzed the data and wrote the first draft of the manuscript, JKH, MFSO, and CDE contributed with data interpretation, HHS supervised the project, CDE and HHS edited the final version of the manuscript.

Declaration of Competing Interest

JKH works for Evonik Operations GmbH, 63457 Hanau, Germany, but the other authors have no conflicts of interest.

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