




# Standardized ileal digestibility of amino acids in four egg products and casein fed to weanling pigs

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## Abstract

An experiment was conducted to test the hypothesis that 1) the standardized ileal digestibility (SID) of amino acids (AA) in pasteurized egg products is sufficient for these ingredients to serve as a high-quality protein source in weanling pig diets; and 2) the SID of AA in trypsin-inhibitor-free egg protein does not differ from that of casein and is greater than the SID of AA in egg products containing residual trypsin inhibitor activity (TIA). Five diets that included raw egg powder (REP), dry-pasteurized egg powder (DPE), liquid pasteurized egg powder (LPE), trypsin inhibitor-free egg powder (TFE), or casein as the only source of AA were formulated. A nitrogen-free diet was also formulated to determine basal endogenous losses of crude protein (CP) and AA. Twelve weanling pigs (initial body weight: 11.99 ± 0.53 kg) with a T-cannula in the distal ileum were allotted to a replicated 6 × 6 Latin square design with six diets and six periods of 7 d. Ileal digesta samples were collected on d 6 and 7 of each period and were analyzed for CP and AA. Results indicated that no differences were observed in SID of CP or AA between REP and DPE or LPE, except that LPE had greater ( $P < 0.05$ ) SID of Lys than REP. The SID of most AA was greater ( $P < 0.05$ ) in TFE and casein compared with REP, DPE, and LPE, indicating that the residual TIA in REP, DPE, and LPE reduced AA digestibility. However, the SID of Lys, Cys, and Ser was not different between TFE and LPE. The SID of His, Lys, Asp, and Ser was less ( $P < 0.05$ ) in TFE than in casein, whereas the SID of other AA and CP in TFE was not different from that in casein. In conclusion, all egg products had excellent SID of CP (>82.0%) and AA (>80.0%), but only when TIA were almost completely eliminated, as in TFE, were the SID of AA in egg protein not different from the SID of AA in casein. Overall, egg products can serve as effective sources of AA in diets for young pigs.

## Lay Summary

During the transition from milk to solid feed, the digestive system of young pigs is not yet fully developed. At this stage, the provision of highly digestible protein sources is critical, as inadequate protein digestion can disturb gastrointestinal function. Eggs contain high-quality protein, but raw eggs contain natural trypsin inhibitors that interfere with protein digestion. Heat treatments such as pasteurization are often applied to reduce microbial load as well as reduce these antinutritional factors to improve amino acid (AA) digestibility. In this experiment, four egg products were tested: raw egg powder, dry-pasteurized egg powder, liquid-pasteurized egg powder, and trypsin inhibitor-free egg powder. Casein, a milk-derived protein characterized by its high digestibility, was also used. Results demonstrated that all egg products had high digestibility for most AA. However, trypsin inhibitor-free egg had protein and AA digestibility values that were not different from values obtained for casein, whereas other pasteurized egg powders had slightly lower values. These observations demonstrate that targeted removal of trypsin inhibitors is more effective in enhancing AA digestibility than mild thermal processing alone.

**Keywords** amino acids, digestibility, egg protein, heat treatment, trypsin inhibitor activity

**Abbreviations:** AA, amino acids; AID, apparent ileal digestibility; CP, crude protein; DPE, dry-pasteurized egg powder; LPE, liquid pasteurized egg powder; REP, raw egg powder; SID, standardized ileal digestibility; TFE, trypsin inhibitor-free egg powder; TIA, trypsin inhibitor activity

## Introduction

Weanling pigs experience significant physiological stress during the transition from milk to solid feed, in part due to an

underdeveloped gastrointestinal tract. At weaning, digestive enzyme secretion and absorptive capacity are still immature, which can reduce the efficiency of nutrient absorption (Pluske et al. 1997; Lallès et al. 2007). Therefore, inclusion of highly

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digestible protein sources in diets for young pigs is critical to support optimal growth and development (Lallès et al. 2007). Feeding poorly digestible proteins may result in an excess of undigested nitrogen reaching the hindgut, which can promote the proliferation of pathogenic bacteria and increase the risk of post-weaning diarrhea (Goodband et al. 2014). To minimize this risk and enhance nutrient absorption, it is essential to formulate diets based on protein sources with high digestibility and low antigenicity (Song et al. 2010).

Animal proteins generally provide high-quality protein with a balanced amino acid (AA) profile, but some animal proteins, such as meat and bone meal, meat meal, and fish meal, may contain connective tissue, high concentrations of minerals, variability in protein quality due to processing, or an imbalance among specific AA, which results in low digestibility of AA (Gottlob et al. 2006; Kong et al. 2014). However, egg proteins have a favorable AA profile and have high digestibility of AA (Fanelli et al. 2024) and may be beneficial in diets for young animals (Ruxton et al. 2010). Various egg products are available for feed use, differing in ingredient composition and processing methods, which may influence nutrient composition and AA digestibility. Raw whole eggs typically contain 1,510 kcal/kg gross energy, 12.5% protein, 11.2% fat, and several vitamins and minerals (Ruxton et al. 2010). Approximately 80% of the egg albumen is composed of proteins that contribute to the physicochemical properties of the egg. Some of these proteins include ovomucoid, ovostatin, ovomucoid, and cystatin (Miranda et al. 2015). These proteins may inhibit proteases, including trypsin and chymotrypsin in humans and pigs (Imondi and Bird 1973; Mine and Yang 2008). The structural characteristics of albumen proteins are expected to reduce protein digestibility in raw eggs, but heat denaturation alters the conformation of these proteins, preventing the inhibition of digestive enzymes. Indeed, heat treatment increased in vitro protein digestibility of egg albumen from 40% to 80% (Schmidt et al. 2007), and the standardized ileal digestibility (SID) of AA in heat-treated or cooked eggs is between 80% and 97% (Heo et al. 2012; Fanelli et al. 2024).

Casein is a milk protein that does not contain antinutritional factors, has a well-characterized AA profile and SID values for most indispensable AA that approach 100% (Stein et al. 2007; Park et al. 2018). Therefore, casein can serve as a comparative protein for evaluating the digestibility of experimental ingredients such as egg products subjected to thermal processing. To our knowledge, however, there is no published information about the SID of AA in pasteurized egg protein devoid of trypsin inhibitor activity (TIA) and other pasteurized heat-treated egg products compared with raw eggs or casein when fed to weanling pigs, and it is not known to which extent mild heat processing or removal of trypsin inhibitors will increase the SID of AA in eggs. Therefore, the objectives of this experiment were to test the hypothesis that 1) pasteurized egg products are high-quality proteins when fed to weanling pigs; and 2) the SID of AA in trypsin-inhibitor-free egg protein does not differ from the SID of AA in casein and is greater than the SID of AA in egg products containing residual TIA.

## Material and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this

experiment before animal work was initiated. Pigs were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

## Dietary treatments

Four different egg products (Symrise Pet Food North America, Hodges, SC, USA), including raw egg powder (REP), dry-pasteurized egg powder (DPE), liquid pasteurized egg powder (LPE), and trypsin inhibitor-free egg powder (TFE) were prepared (Table 1). All egg powders were spray-dried at Dahmes Stainless Inc. (New London, MN, USA) using a Bustle style spray-dryer and Spraying Systems Co. SPRAYDRY NOZZLES = SK Serious 54/21=54 orifice 21 core. To produce DPE, samples were stored in a heated room at 60 °C for 168 h after drying. To produce LPE, liquid whole eggs were heated to 60 °C for 30 min before drying, and TFE was produced by heating liquid whole eggs to 60 °C with pH raised to 9 for 2 h. The level of TIA in egg products was as follows (mg/g): REP, 22.6; DPE, 16.7; LPE, 13.5; and TFE, 2.3, with TIA in TFE reduced by approximately 90.0% compared with REP.

Six experimental diets were prepared (Tables 2 and 3). Five diets included each source of egg product or casein as the sole source of crude protein (CP) and AA. A nitrogen-free diet was

Table 1 Analyzed nutrient composition of ingredients (as-fed basis).<sup>1</sup>

Item	REP	DPE	LPE	TFE	Casein
Dry matter, %	96.16	94.79	90.02	95.89	89.43
Crude protein, %	56.58	51.75	47.12	52.21	86.52
Acid-hydrolyzed ether extract, %	31.85	31.20	35.44	33.10	0.69
Ash, %	4.99	5.87	4.75	7.90	1.62
Trypsin inhibitor activity, mg/g	22.60	16.70	13.50	2.31	–
Indispensable amino acids, %					
Arginine	3.45	3.20	2.84	3.04	3.25
Histidine	1.55	1.43	1.28	1.33	2.63
Isoleucine	3.26	3.05	2.67	2.77	4.81
Leucine	4.96	4.56	4.08	4.26	8.29
Lysine	4.34	4.13	3.66	3.68	7.13
Methionine	1.98	1.81	1.56	1.60	2.48
Phenylalanine	3.33	3.02	2.64	2.74	4.57
Threonine	2.70	2.42	2.22	2.37	3.66
Tryptophan	0.86	0.80	0.67	0.77	1.20
Valine	4.03	3.66	3.23	3.40	5.87
Dispensable amino acids, %					
Alanine	3.32	3.03	2.74	2.81	2.71
Aspartic acid	5.75	5.11	4.62	4.92	6.32
Cysteine	1.48	1.17	1.06	1.08	0.35
Glutamic acid	6.97	6.37	5.70	5.84	19.86
Glycine	2.05	1.86	1.67	1.74	1.65
Proline	2.21	2.04	1.88	1.91	9.31
Serine	3.70	3.45	3.08	3.25	4.19
Tyrosine	2.26	2.12	1.88	2.00	5.09

<sup>1</sup>REP, raw egg powder; DPE, dry-pasteurized egg powder; LPE, liquid pasteurized egg powder; TFE, free trypsin inhibitor activity egg powder.

**Table 2** Ingredient composition of experimental diets (as-fed basis).<sup>1</sup>

Item, %	REP	DPE	LPE	TFE	Casein	Nitrogen-free
Cornstarch	30.60	31.60	25.60	31.60	43.60	54.64
Test protein	35.00	34.00	40.00	34.00	21.00	—
Lactose	12.00	12.00	12.00	12.00	12.00	12.00
Sucrose	15.00	15.00	15.00	15.00	10.00	20.00
Soybean oil	—	—	—	—	6.00	5.00
Ground limestone	0.30	0.30	0.30	0.30	1.00	0.40
Dicalcium phosphate	1.80	1.80	1.80	1.80	1.10	2.16
Cellulose	4.00	4.00	4.00	4.00	4.00	4.00
Magnesium oxide	—	—	—	—	—	0.10
Potassium carbonate	—	—	—	—	—	0.40
Sodium chloride	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
Vitamin-mineral premix <sup>2</sup>	0.50	0.50	0.50	0.50	0.50	0.50

<sup>1</sup>REP, raw egg powder; DPE, dry-pasteurized egg powder; LPE, liquid pasteurized egg powder; TFE, free trypsin inhibitor activity egg powder.

<sup>2</sup>The vitamin-micromineral premix provides the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D<sub>3</sub> as cholecalciferol, 1,660 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B<sub>12</sub>, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

**Table 3** Analyzed nutrient composition of experimental diets (as-fed basis).<sup>1</sup>

Item, %	REP	DPE	LPE	TFE	Casein	Nitrogen-free
Dry matter	94.98	94.27	93.04	94.27	93.09	93.95
Crude protein	19.03	17.34	18.48	16.56	18.79	0.47
Acid-hydrolyzed ether extract	8.41	9.28	13.94	9.79	6.02	3.22
Ash	4.64	4.20	4.57	4.49	2.81	3.37
<b>Indispensable amino acids</b>						
Arginine	1.13	1.11	1.11	0.93	0.65	0.01
Histidine	0.47	0.46	0.47	0.39	0.54	0.01
Isoleucine	1.06	1.06	1.04	0.85	1.00	0.01
Leucine	1.65	1.64	1.64	1.36	1.72	0.02
Lysine	1.46	1.47	1.47	1.15	1.51	0.02
Methionine	0.64	0.67	0.63	0.53	0.50	0.00
Phenylalanine	1.06	1.06	1.03	0.85	0.93	0.01
Threonine	0.86	0.85	0.87	0.73	0.77	0.01
Tryptophan	0.29	0.28	0.27	0.27	0.25	0.02
Valine	1.28	1.27	1.26	1.04	1.21	0.01
<b>Dispensable amino acids</b>						
Alanine	1.12	1.11	1.12	0.91	0.57	0.01
Aspartic acid	1.90	1.88	1.89	1.60	1.33	0.01
Cysteine	0.44	0.43	0.44	0.36	0.09	0.00
Glutamic acid	2.51	2.48	2.45	2.04	4.18	0.03
Glycine	0.65	0.64	0.65	0.54	0.35	0.01
Proline	0.70	0.70	0.70	0.59	1.95	0.01
Serine	1.21	1.21	1.19	1.00	0.93	0.01
Tyrosine	0.66	0.67	0.68	0.55	0.88	0.01

<sup>1</sup>REP, raw egg powder; DPE, dry-pasteurized egg powder; LPE, liquid pasteurized egg powder; TFE, free trypsin inhibitor activity egg powder.

also formulated to determine basal endogenous losses of CP and AA. All diets also contained cornstarch, lactose, sucrose, and soybean oil, and 0.40% chromium oxide. Minerals and

vitamins were included in all diets to meet or exceed the estimated nutrient requirements of young pigs (NRC 2012). A sample of each diet was collected at the time of diet mixing.

## Animals, housing, and sample collection

A total of 12 weanling pigs (initial average body weight:  $11.99 \pm 0.53$  kg) that had a T-cannula installed at the distal ileum (Stein et al. 1998) were used. Pigs were allotted to experimental diets using a replicated  $6 \times 6$  Latin square design with six diets and six periods of 7 d. Therefore, there were a total of 12 observations per treatment. Pigs were housed individually in  $1.2 \times 1.5$  m pens equipped with a self-feeder, a nipple waterer, and a fully slatted tribar floor composed of parallel triangle-shaped steel bars designed to ease the flow of manure.

Pigs were fed their respective diets daily at 0700 h, and all pigs were fed their assigned diets at 4% of body weight. Water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The initial 5 d of each experimental period were considered the adaptation period, but ileal digesta were collected on d 6 and 7 from 0700 to 1600 h using standard procedures (Stein et al. 1998). Cannulas were opened at the beginning of collection, and a 225-mL plastic bag was attached to the cannula barrel using a cable tie. Digesta flowing into the bag were collected and bags were replaced whenever they were full or at least once every 30 min. All samples were stored at  $-20^\circ\text{C}$  after collection. At the conclusion of the experiment, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized and finely ground (Lagos and Stein 2019).

## Chemical analysis

Ingredient, diet, and ileal digesta samples were analyzed for dry matter (method 930.15; AOAC Int., 2019) and for nitrogen using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI, USA; method 990.03; AOAC Int., 2019). CP was calculated as analyzed nitrogen  $\times 6.25$ . Ingredient and diet samples were analyzed for ash (method 942.05; AOAC Int., 2019) and for acid-hydrolyzed ether extract by acid-hydrolysis using 3 N HCl (Ankom<sup>HCl</sup>, Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (Ankom<sup>XT15</sup>, Ankom Technology, Macedon, NY, USA). Amino acids (AA) in ingredient, diet, and ileal digesta samples were analyzed on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6 N HCl for 24 h at  $110^\circ\text{C}$  (method 982.30 E(a); AOAC Int., 2019). 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., 2019). Tryptophan was determined after NaOH hydrolysis for 22 h at  $110^\circ\text{C}$  (method 982.30 E(c); AOAC Int., 2019). Diet and ileal digesta samples were also analyzed for chromium using the Inductive Coupled Plasma Atomic Emission Spectrometric method (method 990.08; AOAC Int., 2019), and samples were prepared using nitric acid-perchloric acid (method 968.08D(b); AOAC Int., 2019). All egg products were also analyzed for TIA (method 22-40; AACCI Int., 2006) at the University of Missouri, MO, USA.

## Calculations

Values for apparent ileal digestibility (AID) of CP and AA in each diet were calculated using the following equation (Stein et al. 2007):

$$\text{AID} = [1 - (\text{nutrient}_{\text{digesta}} / \text{nutrient}_{\text{diet}}) \times (\text{Cr}_{\text{diet}} / \text{Cr}_{\text{digesta}})] \times 100,$$

where AID is the AID value of CP or AA (%) in egg protein,  $\text{nutrient}_{\text{digesta}}$  is the concentration of CP or AA in the ileal digesta (dry matter basis),  $\text{nutrient}_{\text{diet}}$  is the CP or AA concentration in the diet (dry matter basis),  $\text{Cr}_{\text{diet}}$  is the chromium concentration in the diet (dry matter basis), and  $\text{Cr}_{\text{digesta}}$  is the chromium concentration in the ileal digesta (dry matter basis).

The basal endogenous flow to the distal ileum of CP and each AA was determined based on the flow obtained after feeding the nitrogen-free diet using the following equation (Stein et al. 2007):

Basal endogenous loss =  $\text{nutrient}_{\text{digesta}} \times (\text{Cr}_{\text{diet}} / \text{Cr}_{\text{digesta}})$ , where the basal endogenous loss of CP or each AA is determined in mg per kg of dry matter intake.

By correcting the AID for the basal endogenous loss of CP or AA, SID was calculated using the following equation (Stein et al. 2007):

$$\text{SID} = \text{AID} + [(\text{basal endogenous loss} / \text{nutrient}_{\text{diet}}) \times 100],$$

where SID is the SID value of CP or AA (%) in egg protein.

## Statistical analysis

The normality of residuals and homogeneity of variances were verified. When these assumptions were not met, data were transformed using the BOXCOX procedure in SAS (version 9.4; SAS Inst. Inc., Cary, NC, USA). The transformation was applied to all variables that did not meet the normality assumption, using a  $\lambda$  value of 3, and assumptions were subsequently re-checked. Outliers were identified and removed after calculation of AID and SID values and prior to ANOVA as values that deviated from the first or third quartiles by  $\pm 3$  times the interquartile range using internally studentized residuals obtained from the PROC MIXED influence diagnostics. As a result, the number of replicates used in the analysis ranged from 9 to 11. Data were analyzed using the PROC MIXED in SAS using the pig as the experimental unit. The statistical model included diet as the fixed effect and pig, period, and square as random effects. Least square means were calculated, and means were separated with the PDIF option using Tukey's adjustment if the model *P*-value was significant. Statistical significance and tendency were considered at  $P < 0.05$  and  $0.05 \leq P < 0.10$ , respectively.

## Results

The AID of CP, His, Lys, Asp, Glu, Ser, and Tyr was greater ( $P < 0.05$ ) in casein than in all egg products, but the AID of CP, His, and Tyr was greater ( $P < 0.05$ ) in TFE compared with REP, DPE, and LPE, and the AID of Lys, Asp, Glu, and Ser was greater ( $P < 0.05$ ) in TFE than in REP and DPE (Table 4). The AID for all other AA was greater ( $P < 0.05$ ) in TFE and casein when compared with the other egg products, with the exception that the AID of Cys was greater ( $P < 0.05$ ) in TFE than in REP, DPE, and casein.

**Table 4** Apparent ileal digestibility of crude protein and amino acids in protein sources.<sup>1</sup>

Item, %	REP	DPE	LPE	TFE	Casein	SEM	P-value
<i>n</i>	9	10	10	11	11	—	—
Crude protein	72.7 <sup>c</sup>	71.2 <sup>c</sup>	75.3 <sup>c</sup>	82.1 <sup>b</sup>	89.5 <sup>a</sup>	1.70	<0.0001
<b>Indispensable amino acids</b>							
Arginine	80.3 <sup>b</sup>	80.9 <sup>b</sup>	84.8 <sup>b</sup>	90.3 <sup>a</sup>	92.0 <sup>a</sup>	1.48	<0.0001
Histidine	71.3 <sup>c</sup>	72.6 <sup>c</sup>	77.6 <sup>c</sup>	85.1 <sup>b</sup>	94.6 <sup>a</sup>	1.63	<0.0001
Isoleucine	76.8 <sup>b</sup>	77.9 <sup>b</sup>	81.7 <sup>b</sup>	90.5 <sup>a</sup>	94.4 <sup>a</sup>	1.62	<0.0001
Leucine	77.7 <sup>b</sup>	78.7 <sup>b</sup>	82.7 <sup>b</sup>	91.5 <sup>a</sup>	95.1 <sup>a</sup>	1.46	<0.0001
Lysine	74.3 <sup>d</sup>	77.2 <sup>c,d</sup>	81.2 <sup>b,c</sup>	83.9 <sup>b</sup>	94.8 <sup>a</sup>	1.46	<0.0001
Methionine	81.2 <sup>b</sup>	82.0 <sup>b</sup>	86.3 <sup>b</sup>	94.9 <sup>a</sup>	96.9 <sup>a</sup>	1.75	<0.0001
Phenylalanine	76.7 <sup>b</sup>	77.5 <sup>b</sup>	82.0 <sup>b</sup>	91.6 <sup>a</sup>	94.7 <sup>a</sup>	1.76	<0.0001
Threonine	69.8 <sup>b</sup>	71.8 <sup>b</sup>	73.6 <sup>b</sup>	80.4 <sup>a</sup>	86.2 <sup>a</sup>	1.66	<0.0001
Tryptophan	74.8 <sup>b</sup>	75.2 <sup>b</sup>	80.9 <sup>b</sup>	92.2 <sup>a</sup>	94.3 <sup>a</sup>	1.51	<0.0001
Valine	75.4 <sup>b</sup>	76.7 <sup>b</sup>	80.8 <sup>b</sup>	89.8 <sup>a</sup>	93.8 <sup>a</sup>	1.58	<0.0001
<b>Dispensable amino acids</b>							
Alanine	74.6 <sup>b</sup>	76.2 <sup>b</sup>	79.8 <sup>b</sup>	87.3 <sup>a</sup>	86.1 <sup>a</sup>	1.65	<0.0001
Aspartic acid	73.5 <sup>c</sup>	74.5 <sup>c</sup>	75.8 <sup>b,c</sup>	81.2 <sup>b</sup>	91.2 <sup>a</sup>	1.59	<0.0001
Cysteine	66.4 <sup>b,c</sup>	70.0 <sup>b,c</sup>	74.2 <sup>a,b</sup>	79.9 <sup>a</sup>	63.1 <sup>c</sup>	2.33	<0.0001
Glutamic acid	75.4 <sup>c</sup>	77.3 <sup>c</sup>	81.5 <sup>b,c</sup>	87.8 <sup>b</sup>	95.7 <sup>a</sup>	1.64	<0.0001
Serine	71.7 <sup>c</sup>	72.6 <sup>c</sup>	74.8 <sup>b,c</sup>	79.0 <sup>b</sup>	92.0 <sup>a</sup>	1.33	<0.0001
Tyrosine	76.7 <sup>c</sup>	78.2 <sup>c</sup>	81.8 <sup>c</sup>	89.9 <sup>b</sup>	95.3 <sup>a</sup>	1.38	<0.0001

<sup>1</sup>REP = raw egg powder; DPE = dry-pasteurized egg powder; LPE = liquid pasteurized egg powder; SEM = standard error of the mean; TFE = free trypsin inhibitor activity egg powder.

<sup>a-d</sup>Means within a row lacking a common superscript letter differ. ( $P < 0.05$ ).

The SID of CP, Arg, Ile, Leu, Met, Phe, Thr, Trp, Val, Ala, Glu, and Tyr was greater ( $P < 0.05$ ) in TFE and casein compared with the other egg products, and TFE and casein also had greater ( $P < 0.05$ ) SID of Cys than REP and DPE (Table 5). The SID of His, Lys, Asp, and Ser was greater ( $P < 0.05$ ) in casein than in all egg products, but TFE had greater ( $P < 0.05$ ) SID of His and Asp compared with REP, DPE, and LPE, and also greater ( $P < 0.05$ ) SID of Lys and Ser than REP and DPE.

## Discussion

In diets for weanling pigs, egg products are considered specialty protein ingredients and can be appropriately compared with specialty soy ingredients and plasma-based proteins, but egg proteins are usually priced lower than plasma proteins. Although this experiment focused on AA digestibility, egg products also have a high concentration of fat and therefore have a greater energy concentration than other specialty proteins. Therefore, inclusion of egg products in diets for pigs will also increase diet energy concentration, which may result in increased feed efficiency.

The analyzed egg-based ingredients varied in CP and total AA concentrations (47.0% to 57.0%), which may be a result of the processing methods used to manufacture each ingredient. Acid-hydrolyzed ether extract was greater in LPE and TFE compared with REP and DPE, which may reflect the differences in liquid pasteurization processes or raw material sourcing and separation prior to drying. The greater ash concentration in TFE may be due to processing steps intended to inactivate TIA, which

involved thermal and pH treatment with inorganic salts (Vagadia et al. 2017).

Among indispensable AA, REP generally had the greatest concentrations among the egg products, likely due to the absence of processing, although concentrations were less than those in casein. The observation that Lys was greater in REP (4.3%) compared with the heated egg products (3.7% to 4.1%) likely reflects protein denaturation and Maillard reactions that can occur during prolonged temperature processing, particularly in DPE, LPE, and TFE (Teodorowicz et al. 2017). However, the Lys: CP ratio among REP, DPE, and LPE was similar (7.7% to 8.0%), whereas in TFE, the Lys: CP ratio was reduced (7.0%). Similarly, dispensable AA such as Ala, Asp, and Ser followed the same trend, with REP having greater concentrations than LPE and TFE. Nevertheless, despite the greater AA concentrations observed in REP, heat treatment is required to eliminate pathogens, and thus the REP used in this experiment does not represent a commercially available ingredient.

The SID of CP and all AA in casein was in agreement with reported values (>98.0%), confirming the validity of the current results and supporting its use as a comparative protein (Deglaire et al. 2009; Cervantes-Pahm and Stein 2010; NRC 2012; Park et al. 2018). The SID of AA (from 76.0 to 87.0%) in REP was in agreement with values reported by Sung et al. (2020). The SID for CP and all AA in DPE, LPE, and TFE were either not different from or greater than in most common animal protein sources (NRC 2012). Specifically, the average SID of AA in LPE (approximately 84.0%) was in agreement with the SID of AA in spray-dried plasma protein, which is considered a high-quality protein for weanling pigs, and the average SID of AA in TFE

**Table 5** Standardized ileal digestibility of crude protein and amino acids in protein sources.<sup>1,2</sup>

Item, %	REP	DPE	LPE	TFE	Casein	SEM	P-value
<i>n</i>	9	10	10	11	11	—	—
Crude protein	82.5 <sup>b</sup>	81.8 <sup>b</sup>	85.1 <sup>b</sup>	93.2 <sup>a</sup>	99.1 <sup>a</sup>	1.70	<0.0001
<b>Indispensable amino acids</b>							
Arginine	86.9 <sup>b</sup>	87.6 <sup>b</sup>	91.4 <sup>b</sup>	98.3 <sup>a</sup>	103.3 <sup>a</sup>	1.48	<0.0001
Histidine	76.3 <sup>c</sup>	77.7 <sup>c</sup>	82.5 <sup>c</sup>	91.1 <sup>b</sup>	98.9 <sup>a</sup>	1.63	<0.0001
Isoleucine	80.2 <sup>b</sup>	81.2 <sup>b</sup>	85.1 <sup>b</sup>	94.7 <sup>a</sup>	97.9 <sup>a</sup>	1.62	<0.0001
Leucine	81.2 <sup>b</sup>	82.2 <sup>b</sup>	86.1 <sup>b</sup>	95.7 <sup>a</sup>	98.4 <sup>a</sup>	1.46	<0.0001
Lysine	77.9 <sup>d</sup>	80.7 <sup>c,d</sup>	84.7 <sup>b,c</sup>	88.4 <sup>b</sup>	98.2 <sup>a</sup>	1.46	<0.0001
Methionine	82.6 <sup>b</sup>	83.3 <sup>b</sup>	87.7 <sup>b</sup>	96.6 <sup>a</sup>	98.7 <sup>a</sup>	1.75	<0.0001
Phenylalanine	80.0 <sup>b</sup>	80.8 <sup>b</sup>	85.3 <sup>b</sup>	95.7 <sup>a</sup>	98.3 <sup>a</sup>	1.76	<0.0001
Threonine	78.0 <sup>b</sup>	80.0 <sup>b</sup>	81.5 <sup>b</sup>	89.8 <sup>a</sup>	95.1 <sup>a</sup>	1.66	<0.0001
Tryptophan	78.4 <sup>b</sup>	78.9 <sup>b</sup>	84.7 <sup>b</sup>	96.1 <sup>a</sup>	98.5 <sup>a</sup>	1.51	<0.0001
Valine	79.0 <sup>b</sup>	80.3 <sup>b</sup>	84.3 <sup>b</sup>	94.2 <sup>a</sup>	97.5 <sup>a</sup>	1.58	<0.0001
<b>Dispensable amino acids</b>							
Alanine	80.3 <sup>b</sup>	81.9 <sup>b</sup>	85.4 <sup>b</sup>	94.2 <sup>a</sup>	97.0 <sup>a</sup>	1.65	<0.0001
Aspartic acid	77.6 <sup>c</sup>	78.7 <sup>c</sup>	79.9 <sup>c</sup>	83.1 <sup>b</sup>	97.1 <sup>a</sup>	1.59	<0.0001
Cysteine	73.4 <sup>c</sup>	77.2 <sup>c</sup>	81.1 <sup>b,c</sup>	88.5 <sup>a,b</sup>	97.0 <sup>a</sup>	2.33	<0.0001
Glutamic acid	79.1 <sup>b</sup>	81.0 <sup>b</sup>	85.2 <sup>b</sup>	92.3 <sup>a</sup>	97.9 <sup>a</sup>	1.64	<0.0001
Serine	76.6 <sup>c</sup>	77.4 <sup>c</sup>	79.7 <sup>bc</sup>	84.9 <sup>b</sup>	98.2 <sup>a</sup>	1.33	<0.0001
Tyrosine	81.4 <sup>b</sup>	82.7 <sup>b</sup>	86.1 <sup>b</sup>	95.4 <sup>a</sup>	98.7 <sup>a</sup>	1.38	<0.0001

<sup>1</sup>REP, raw egg powder; DPE, dry-pasteurized egg powder; LPE, liquid pasteurized egg powder; SEM, standard error of the mean; TFE, free trypsin inhibitor activity egg powder.

<sup>2</sup>Average values of basal ileal endogenous losses (g/kg dry matter intake) were as follows: Crude protein 19.48; Arginine 0.79; Histidine 0.25; Isoleucine 0.37; Leucine 0.61; Lysine 0.54; Methionine 0.10; Phenylalanine 0.37; Threonine 0.74; Tryptophan 0.11; Valine 0.48; Alanine 0.67; Aspartic acid 0.84; Cysteine 0.33; Glutamic acid 0.97; Serine 0.62; and Tyrosine 0.32.

<sup>a-d</sup>Means within a row lacking a common superscript letter differ. ( $P < 0.05$ ).

(approximately 92.0%) was greater than in spray-dried plasma protein (Mateo and Stein 2007; Bailey et al. 2026). This confirms that these pasteurized egg products are high-quality proteins that may be used in diets for weanling pigs, providing digestibility values that are greater than 80.0% for most AA.

The observation that the SID of all AA in DPE or LPE was less than the SID of AA in fried, boiled, or scrambled eggs (Fanelli et al. 2024) is likely due to the fact that the egg products used in this experiment were pasteurized and not cooked. Pasteurization applies to a relatively mild heat treatment to reduce microbial load while preserving the functional properties of eggs, whereas cooking subjects eggs to higher temperatures that induce greater protein denaturation. This enhanced denaturation reduces TIA and exposes peptide bonds, thereby facilitating enzyme access and improving AA digestibility (Hodgkinson et al. 2018). The observation that the SID of all AA in TFE were in agreement with values for fried, boiled, or scrambled eggs (Fanelli et al. 2024) further indicates that reduction in TIA (from 22.6 to 2.3 mg/g) increases SID of AA. Similarly, heat treatment at 110 °C for 10 min in egg by-products was more effective in increasing protein digestibility in rats than spray-drying (Schmidt et al. 2007).

The heat processing used to produce DPE or LPE did not affect the SID of CP or AA when compared with REP (average range of 80.0% to 84.0%). This is in agreement with data demonstrating that spray-dried technical albumen that was stored at 70 °C for 3 d and then fed to weaned pigs had AID of AA that was not

different from egg by-products (Schmidt et al. 2003). This may be because the temperature used to produce DPE or LPE was not high enough or was applied for too short time to inactivate a significant part of the trypsin inhibitors. Although it is well established that heat treatment reduces TIA, the temperature and duration of heating at which inactivation begins remain unclear. In eggs, the primary trypsin inhibitor is ovomucoid, which differs structurally and functionally from the Kunitz and Bowman-Birk inhibitors in soybeans and soybean co-products and therefore may respond differently to thermal processing. This distinction is important when considering effects on AA digestibility, as the extent of ovomucoid inactivation likely contributes to the improvement observed with different thermal processes (Plancken et al. 2004). This hypothesis is supported by the fact that when TIA was reduced in TFE, the SID of CP and all AA increased and was greater than in the other three egg products and not different from casein.

In the present experiment, the egg products were included as the sole protein source in diets for weanling pigs to ensure requirements for protein were met while determining differences in AA digestibility. However, inclusion rates exceeding 30% are not representative of practical feeding scenarios. Under commercial conditions, pasteurized egg products would more likely be included at levels of 3% to 5% of the diet for young pigs. At such inclusion rates, the contribution of TIA from DPE and LPE would likely be comparable to that in the diet containing TFE in the current experiment (Table S1), and SID of AA in

DPE and LPE may, therefore, be greater than measured in this experiment and closer to the SID of AA determined for TFE. Conversely, if egg powders were included at levels greater than 5%, lower AA digestibility, as observed with DPE and LPE in the present study, would need to be considered in diet formulation. These observations support the potential use of pasteurized egg products as a high-quality protein source in practical diets for weaning pigs, but further research is needed to validate this hypothesis under commercial feeding conditions, particularly given that TFE is not yet commercially available.

## Conclusion

All egg products used in this experiment had high SID of CP and AA. However, only when egg TIA was reduced were the SID of AA comparable with values for casein. This was not the case for commercially available products such as DPE and LEP that had less SID of AA than casein, which was likely due to the residual TIA. Results indicate that targeted reduction of TIA is more effective in improving the SID of AA than mild thermal treatments alone. Regardless, egg products can be used as a source of AA in diets for young pigs.

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## Author contributions

Natalia S. Fanelli (Data curation, Formal analysis, Investigation, Methodology, Writing—original draft), Minoy Cristobal (Investigation, Methodology, Resources, Writing—original draft), Su A Lee (Formal analysis, Investigation, Project administration, Supervision, Writing—review & editing), Yi-Chi Cheng (Conceptualization, Funding acquisition, Resources, Writing—review & editing), Ingmar Middelbos (Conceptualization, Funding acquisition, Methodology, Resources, Writing—review & editing), and Hans H. Stein (Conceptualization, Funding acquisition, Project administration, Resources, Writing—review & editing)

## Supplementary data

Supplementary data is available at *Journal of Animal Science* online.

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## Data availability

All data from this experiment are included in the manuscript.

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