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Correlation of an Immobilized Digestive Enzyme Assay with Poultry True Amino Acid Digestibility for Soybean Meal

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ABSTRACT The immobilized digestive enzyme assay (IDEA) was run on 6 soybean meal (SBM) samples and compared with true amino acid digestibility (TAAD) content, as determined using cecectomized roosters. The IDEA values were excellent TAAD predictors as evidenced by R^2 values of 0.90 and 0.88 for lysine and cystine, respectively. The original IDEA took 2.5 d to run, and therefore we modified the protocol to reduce the time to 18 h. This modified IDEA procedure was run on 17 SBM samples, and IDEA values were shown to be excellent predictors of TAAD content. This IDEA SBM kit was validated by predicting the TAAD of 5 SBM not included in the 17-sample set above and the comparison of the predicted vs. determined TAAD. Finally, the IDEA SBM kit was used to compare the predicted TAAD of 338 SBM samples from around the world. The predicted lysine digestibility on the world survey samples ranged from 70.6 to 95.5% with an average of ~89%, and the ranges and means of the other amino acid digestibilities were also calculated.

Key words: protein digestibility, in vitro assay, in vivo/in vitro correlation, soybean meal

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INTRODUCTION

The cost of feed represents >65% of the bird arriving at a US processing plant (July 2005 Agri Stats); therefore a more precise knowledge of the nutritional value of feed ingredients would enable the formulation of diets that more closely meet amino acid requirements. In vitro and in vivo techniques exist to monitor the quality of proteins in feedstuffs. One of the most common in vivo methods to determine true amino acid digestibility (**TAAD**) is the precision-fed cecectomized rooster assay (Fernandez and Parsons, 1996). The time and cost required for this analysis limits its utility on a regular basis. The need for rapid in vitro assays of protein quality is important for nutrition-based formulation of feeds (Ravindran and Bryden, 1999; Boisen, 2000). Many in vitro assays have been used with varying degrees of success to evaluate protein ingredient quality, including the urease assay (AOAC, 1980), potassium hydroxide solubility (Parsons et al., 1991), nitrogen solubility index (AOCS official method Ba 11-65), the protein dispersibility index (AOCS recommended practice Ba 10a-05), and pepsin digestibility (AOAC official method 971.09). We have developed a system of immobilized enzymes for protein digestibility determination adapted from a technique pioneered for human foodstuffs (Porter et al., 1984; Chang et al., 1990). Their original system used pepsin in a low pH digestor followed by neutralization and digestion with chymotrypsin, trypsin, and intestinal peptidase in a second digestor for a total assay time of 2.5 d. Our goal was to provide a more rapid and accurate prediction of amino acid digestibility for soybean meal (**SBM**). This paper describes the optimized immobilized digestive enzyme assay (**IDEA**) for SBM in a kit format with a single-use digestor configuration and the correlation of IDEA digestion with in vivo TAAD. The IDEA kit described takes ~1 d to run and provides a good prediction of SBM in vivo TAAD obtained using the precision-fed rooster.

MATERIALS AND METHODS

Glass Derivatization

Controlled-pore glass (2,000-Å pore, 80-120 mesh; Sigma Chemical Co., St. Louis, MO) was acid-cleaned and silanized and succinylated as described by Swaisgood et al. (1976).

Enzyme Immobilization

Trypsin, chymotrypsin, and intestinal peptidase were immobilized using the sequential activation/immobilization procedure of Janolino and Swaisgood (1982). Trypsin (porcine; Sigma Chemical Co.) was immobilized by treating a solution containing 6 mg/mL of trypsin (10 mL) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (10

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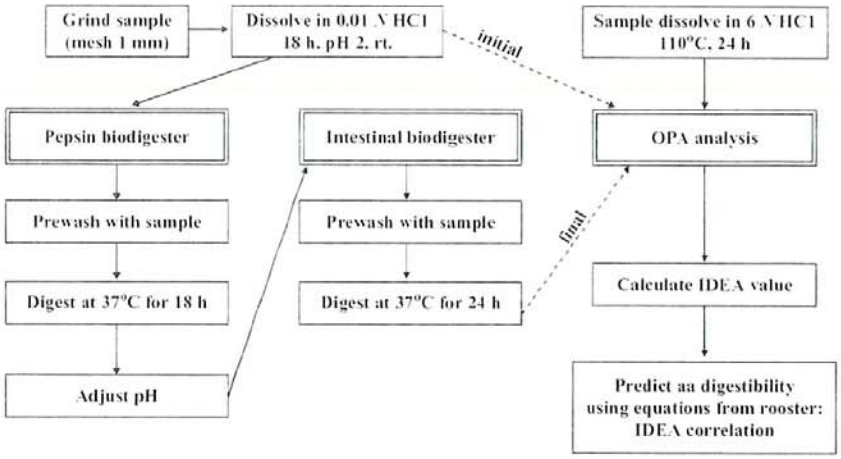

Figure 1. Original immobilized digestive enzyme assay (IDEA) scheme. OPA = *o*-phthaldialdehyde.

m*M*) with succinamidopropyl-glass beads (3 mL) for 20 h at 4°C. Chymotrypsin (porcine; Sigma Chemical Co.) was immobilized by an identical procedure. Activity for the immobilized trypsin was 151 U/g of beads using *p*-tosyl-L-arginine methyl ester (Sigma Chemical Co.) as substrate and that for chymotrypsin was 49.6 U/g of beads using benzoyltyrosine ethyl ester (Sigma Chemical Co.). Activity was measured by continuously monitoring the increase in absorbance resulting from the hydrolysis of the substrate (1 m*M* *p*-tosyl-L-arginine methyl ester/0.5 m*M* benzoyltyrosine ethyl ester in 20 m*M* phosphate buffer at pH 7.5 and 25°C). Intestinal peptidase (porcine; Sigma Chemical Co.) was partially purified by DEAE-Sephacryl and immobilized on aminopropyl-glass by mixing enzyme with 10 m*M* 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide at 4°C (Porter et al., 1984). Activity for the immobilized peptidases was 0.32 U/g of beads using L-leucine-*p*-nitroanilide (Sigma Chemical Co.) as substrate. Activity was determined by continuously monitoring the increase in absorbance at 405 nm resulting from the hydrolysis of the substrate (0.8 m*M* in 50 m*M* phosphate buffer at pH 7.2 and 25°C).

Original IDEA Assay

A schematic of the original IDEA procedure used to assay feedstuff quality is shown in Figure 1 and requires a sample assay total time of ~2.5 d. This procedure is a stepwise acid solubilization, pepsin digestion, neutralization, trypsin, chymotrypsin, and intestinal peptidase digestion followed by analysis of newly exposed α-amino groups.

All feed ingredient samples were ground to a fine powder that was able to pass through a 1-mm mesh screen. Sample solutions were made by dissolving the ground sample in a) 0.01 *N* HCl, 0.1% NaN_3, pH 2.2 for the original IDEA assay or b) 50 m*M* sodium phosphate buffer, pH 7.50 for the IDEA SBM kit assay to give an approximate protein concentration of 2 mg/mL or less.

The stomach (pepsin) bioreactor contained 1.5 mL of immobilized pepsin in an econo-pack column (Biorad, Hercules, CA). Prior to use, each reactor was washed with 10 mL of sample solution, and the washings were discarded. Digestion was carried out by mixing 15 mL of sample with immobilized enzyme on a rotator (20 orbits per min, 23° angle fixed tilt) for 18 h at 37°C. The pepsin-treated sample was collected and adjusted to pH 7.5 by addition of solid Na_2HPO_4.

The intestinal bioreactor contained 0.2 mL immobilized trypsin, 0.3 mL chymotrypsin, and 1.0 mL of intestinal peptidase in another econo-pack column. The bioreactor was washed with 5 mL of the pepsin hydrolysate and the washing discarded. The remaining sample (5 mL) was incubated for 24 h at 37°C.

Digestibility for the original IDEA assay was defined as the fraction of the total peptide bonds hydrolyzed by the 2 bioreactors. α-Amino groups were quantified by reaction with *o*-phthaldialdehyde (OPA). Digestibility was calculated using the relationship

$$\text{Digestibility} = [A_{340}(\text{final}) - A_{340}(\text{initial})] \,/\, [A_{340}(\text{acid}) - A_{340}(\text{initial})],$$

where A_{340} (final) is the absorbance of the OPA assay of the final hydrolysate, A_{340} (initial) is for the undigested sample, and A_{340} (acid) for initial samples completely hydrolyzed in 6 *N* HCl, 110°C for 24 h.

IDEA SBM Kit

The original IDEA scheme took ~2.5 d so we modified the assay to reduce time, and the optimized SBM kit scheme is shown in Figure 2. The shaded steps shown in Figure 2 represent the eliminated steps from the original IDEA assay of Figure 1, and italics represent step modifications. The optimized IDEA SBM kit eliminated the stomach digestor and uses only the intestinal digestor. Finally, the original IDEA assay determined the total pep-

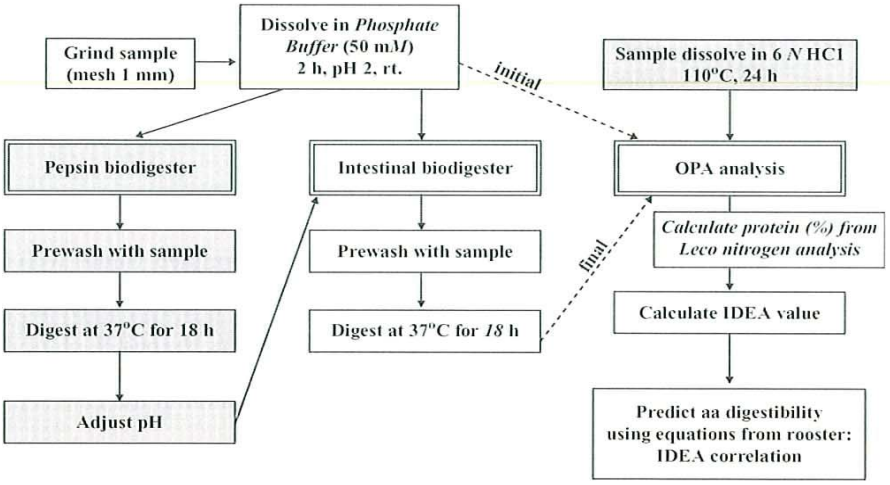

Figure 2. The immobilized digestive enzyme assay (IDEA) soybean meal kit scheme—steps eliminated from original IDEA are indicated by shading and changes indicated by italics. OPA = *o*-phthaldialdehyde.

tide bonds in the sample using acid hydrolysis (24 h), whereas the optimized IDEA SBM kit uses nitrogen combustion analysis (15 min) to calculate a percent protein. Calculation of the IDEA value for both assays uses a parameter in the denominator to provide sample protein comparability. The IDEA SBM kit uses the following procedure as shown in Figure 2. The SBM samples are ground as above, and ~800 mg (duplicate samples) is added to sufficient solubilization buffer to give a 16 mg/mL solution. The solubilization buffer is 50 m*M* phosphate buffer containing 0.1% sodium azide, pH 2.0. Each sample is mixed in a beaker with stir bar for 2 h at room temperature. Following this step, the solution pH is adjusted to 7.50 by the dropwise addition of NaOH (12.5 *N*). Then, remove 1 mL of this pH-adjusted sample into a centrifuge tube for OPA analysis (initial value sample). Digestion is carried out by transferring 250 μL of the pH-adjusted solution above into the digestor tube (2 mL centrifuge tube containing 100 mg of the intestinal enzyme mixture described above). The digestor is then mixed on an end-to-end rotator for 18 h at 37°C (incubator or water bath). Following the digestion step, the digestor is removed from the rotator, the enzyme beads allowed to settle by gravity, and a sample (final value sample) removed for OPA analysis as described below. The optimized IDEA SBM kit reduced the overall assay time from ~2.5 d to <1 d (Figure 2).

Protein Digestion Quantification

Digestion was quantified by the reaction of α-amino groups with OPA (Porter et al., 1984). The OPA reagent was prepared by combining the following components and diluting to 100 mL with water: 50 mL of 0.1 *M* sodium borate, 80 mg of OPA dissolved in 2 mL of 95% ethanol, 200 μL of 2-mercaptoethanol, and 5 mL of 20% sodium dodecyl sulfate. An aliquot (10-30 μL) of sample was added to OPA reagent (1 mL) and incubated for 2 min at room temperature after which the absorbance (340_{nm}) was measured (Cary 3e UV/Vis: Varian Associates, Sunnyvale, CA). The optimized IDEA SBM kit value was calculated as follows:

$$\text{IDEA value} = [A_{340}(\text{final}) - A_{340}(\text{initial})]/\text{percent protein},$$

where A_{340} (final) is the absorbance of the OPA assay of the final hydrolysate, A_{340} (initial) is for the undigested solubilized sample, and percent protein was calculated from the measured combustible nitrogen value (Leco FP 528; Leco Corporation, St. Joseph, MI).

Soybean meal samples were obtained from commercial sources collected in different years (2001-2005) and different countries. These different countries are identified in the text but cannot be taken as country of origin. In addition, raw soyflakes were heated in an autoclave (121°C at 16 psi) for 0, 18, 30, and 36 min. These samples with analyzed CP (43 to 49%) were used in the in vitro assays in addition to the in vivo true digestibility in the cockerel described below.

Table 1. Comparison of immobilized digestive enzyme assay (IDEA) predicted and determined in vivo digestibilities of different soybean meals (SBM)

SBM sample	IDEA value	True digestible lysine	True digestible cystine
1	0.238	76.6	63.2
2	0.287	85.7	81.8
3	0.356	86.2	84.8
4	0.406	92.0	90.7
5	0.420	92.2	92.0
6	0.426	96.0	93.0

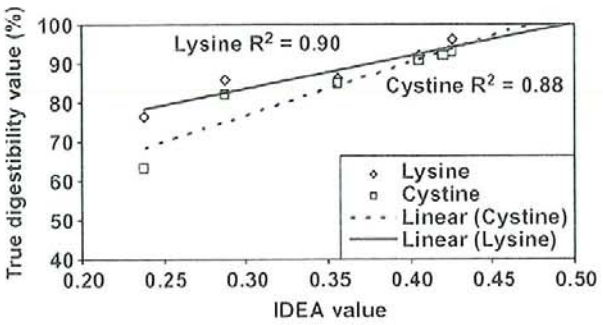

Figure 3. Comparison of immobilized enzyme digestion assay (IDEA) values and in vivo true amino acid digestibilities for soybean meal lysine and cystine.

TAAD Assay

Mature Single Comb White Leghorn roosters approximately 50 wk of age were used. The birds were housed in an environmentally regulated room and kept in individual cages with raised wire floors and subjected to a photoperiod of 16L:8D daily. Feed and water were supplied for ad libitum access before the start of the experiments. Cecectomy was performed according to the procedure of Parsons (1985) when the birds were 25 wk of age. All roosters were given at least 8 wk to recover from the surgery prior to being used in experiments. The assay procedure was that described by Sibbald (1979), with some minor modifications described by Parsons (1985). Following a 24-h period without feed, roosters were given 30 g of the test material via crop intubation. Additional roosters were deprived of feed throughout the experimental period to measure endogenous levels. Three roosters were assigned to each treatment. A plastic tray was placed under each cage and excreta were collected quantitatively for 48 h after crop intubation. The excreta samples were lyophilized, weighed, and ground to pass through a 60-mesh screen. Amino acid concentrations were determined (2 replicates of each individual sample of excreta using AOAC official method 982.30), and true digestibilities of amino acids were calculated according to the method of Sibbald (1979), as modified by Parsons et al. (1991).

Statistical Methods

The IDEA determined values were plotted vs. TAAD in vivo values and linear least squares regression (PROC GLM of SAS; SAS Institute, 2003) to provide linear equations which describe the best fit of the data for each amino acid, and R^2 values which indicate how well the equations describe the variation in the data set.

RESULTS AND DISCUSSION

Original IDEA Assay and Optimization to IDEA SBM Kit Assay

A set of 6 SBM samples was run through the original IDEA and the rooster assay. The IDEA values were determined independently and prior to the receipt of the in vivo digestibility measurements. The calculated IDEA values and determined true digestibility for lysine and cystine are given in Table 1, and these data are compared in Figure 3. Linear least squares regression of IDEA and TAAD data gave equations describing the best fit lines for the SBM digestible lysine and cystine with R^2 values of 0.90 and 0.88, respectively. These R^2 values indicate that the linear equations predict 90 and 88% of the variation of the respective SBM lysine and cystine seen with these 6 samples. These results suggested that the original IDEA assay prediction equations derived from true digestibility correlation were able to predict most of the variation in digestibility seen in the SBM samples tested.

Table 2. True amino acid digestibility coefficients and immobilized digestive enzyme assay (IDEA) values for 17 soybean meal (SBM) samples

SBM sample	Lys	Cys	Arg	Met	Thr	Val	Ile	Leu	Tyr	Phe	His	Trp	IDEA
1	95.0	90.6	94.2	97.1	94.6	95.3	96.3	95.9	96.8	95.9	95.3	97.4	0.863
2	87.7	91.6	90.9	87.7	89.9	87.4	89.7	89.5	92.8	89.6	91.5	96.0	0.833
3	71.4	60.3	76.3	63.5	69.0	63.6	66.1	68.8	69.9	69.4	75.4	63.7	0.442
4	73.3	69.9	81.6	72.8	73.3	69.1	72.1	73.1	77.4	74.7	78.6	77.2	0.551
5	89.1	83.5	91.9	87.2	85.1	84.2	87.2	86.7	90.4	87.7	90.8	92.1	0.684
6	90.4	93.8	92.6	92.6	92.7	91.5	93.7	93.2	96.1	93.6	93.3	96.4	0.860
7	85.4	86.2	91.6	85.0	86.8	85.7	88.2	87.7	90.3	88.8	89.2	93.3	0.731
8	90.9	89.6	93.3	90.6	91.1	89.4	92.3	91.8	94.2	91.9	92.7	95.1	0.853
9	88.0	90.9	93.1	90.5	89.1	91.5	93.8	92.8	94.3	92.6	91.8	96.3	0.788
10	86.6	91.7	93.2	93.6	93.4	93.7	95.3	94.7	96.0	94.3	93.1	97.8	0.774
11	88.0	86.0	89.0	92.7	87.6	92.4	92.1	91.9	92.4	92.8	89.9	93.0	0.775
12	87.1	84.5	88.7	90.7	86.6	90.8	90.4	90.1	91.8	91.4	87.9	93.6	0.835
13	87.2	86.0	91.4	91.6	88.0	91.4	91.4	91.6	92.8	92.7	88.9	94.4	0.787
14	92.6	88.1	94.1	94.2	91.9	94.3	96.6	95.0	96.8	94.7	92.1	99.4	0.891
15	91.9	81.2	95.0	93.4	90.9	90.0	95.1	94.2	94.8	93.3	93.8	96.1	0.899
16	93.6	92.2	94.8	94.6	93.9	94.3	97.0	96.0	98.0	96.0	94.3	95.6	0.873
17	90.1	87.8	92.9	89.8	88.8	88.7	91.0	89.7	90.0	91.6	90.6	92.5	0.795

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Table 3. Relationship (R^2) between soybean meal (SBM) immobilized digestive enzyme assay (IDEA) and determined in vivo amino acid digestibility

Amino acid	R^2 for SBM IDEA kit vs. in vivo	In vivo digestibility range (%)
Lys	0.86	71.4–95.0
Met	0.88	63.5–97.1
Cys	0.73	60.3–93.8
Thr	0.88	69.0–94.6
Arg	0.81	76.3–95.0
Val	0.86	63.6–95.3
Ile	0.90	66.1–97.0
Leu	0.89	68.8–96.0
His	0.83	75.4–95.3
Tyr	0.89	69.9–98.0
Phe	0.88	69.4–96.0
Asp	0.91	71.0–93.7
Ser	0.88	68.5–97.4
Glu	0.90	77.2–95.9
Pro	0.86	74.0–98.9
Ala	0.88	64.7–92.5
Trp	0.85	63.7–99.4

IDEA Kit Standard Curve Correlation with in Vivo Amino Acid Digestibility Determination

A set of 17 SBM samples were run through the IDEA SBM kit and the rooster assay. The IDEA values were determined independently and prior to the receipt of the in vivo digestibility measurements. The calculated IDEA SBM kit values and determined true digestibility for 17 amino acids is given in Table 2. The relationship of the 17-sample SBM standard curve in vivo and IDEA values is shown in Table 3. Linear regression analysis of the data from the in vivo and in vitro results gave R^2 values ranging from 0.73 to 0.91. The amino acid digestibility prediction range of the IDEA SBM kit is dictated by the in vivo data and is also shown in Table 3. This is important because when SBM samples are run through the IDEA SBM kit and predict amino acid digestibility values outside the standard curve in vivo data set, we can request samples so they can be run through the rooster digestibility assay to be added to our standard curve and make our predictions more accurate. The intraassay CV of a single sample of SBM for determination of the IDEA SBM kit value was determined to be 1.4% (data not shown).

Validation of the IDEA Kit Predictions of SBM Amino Acid Digestibilities

A validation set of 5 SBM samples not included in the above standard curve determination were run through the IDEA kit, and predicted amino acid digestibilities and then TAAD were determined in roosters. Comparison of the individual sample IDEA calculated and in vivo true amino acid digestibilities is shown in Table 4. The difference in IDEA predicted and determined true digestibilities is greatest for the amino acid cystine, with a mean difference percentage value of 7.24, in agreement with this amino acid having the lowest R^2 value in the standardization sample set (Table 3). United States Patent 6,750,035 has been granted for the invention of this technology (Schasteen and Wu, 2004).

SBM World Survey Using IDEA

The power of the IDEA kit to rapidly survey protein ingredient predicted digestibility is shown in Table 5. Samples (n) of SBM were collected out of Novus International's sample archives from North America (233), Latin America (43), the European Union (28), and Asia (34) from 2001 to 2005. Samples were collected in each world area and stored at 4°C. Although we cannot authenticate the nation of origin for each sample, they provide an indication of the SBM protein quality found in each particular world area during the sampling period. The time and cost of running this many in vivo cockerel assays is prohibitive; however, this number of IDEA kit analyses

Table 4. Validation set of soybean meal (SBM) immobilized digestive enzyme assay (IDEA) predicted amino acid digestibility

Amino acid	SBM 1		SBM 2		SBM 3		SBM 4		SBM 5		Mean difference %
	True	IDEA	True	IDEA	True	IDEA	True	IDEA	True	IDEA	
Lys	89	89.5	86.5	87.5	85.9	88.2	82.3	85.3	83.8	86.2	2.18
Met	91.5	87.3	91.5	83.1	89.9	89.2	85.6	85.1	92.1	86.4	4.26
Cys	86	84	85.7	81	82.2	89.4	72.6	84.4	89.1	86.1	7.24
Thr	88.2	86.7	87.8	84	87.5	88	86.5	84.5	87.7	85.6	2.26
Arg	89.8	91.7	91.1	89.8	94.7	91.6	93.5	89.1	89.9	89.9	2.30
Val	91.5	87.6	91	84.3	87.6	87.5	87.1	83.2	86.6	84.6	3.71
Ile	90.9	89.2	91.1	85.9	91.3	89.5	90.7	85.4	91.2	86.7	4.07
Leu	90.8	89.7	91.3	86.9	91.7	89.5	90.9	85.8	90.4	87.1	3.54
His	89	90.3	88.4	88	86.8	90.5	83.6	87.7	87.7	88.6	2.42
Tyr	91.8	88.9	92.2	85.3	93.8	93.1	94.5	89.3	93.2	90.5	3.96
Phe	92.1	92.1	92.6	89.7	93.3	90.8	93.2	87.3	92.1	88.5	3.21
Asp	91	88.8	89.6	96.4	87.6	88.1	84.5	85.1	89.6	86.1	2.24
Ser	91.7	91.4	91.5	88.8	87.9	90.8	88.2	87.1	87.8	88.3	1.68
Glu	93.3	92.3	92.6	90.6	91.9	94.4	90.3	89	91.9	89.8	1.50
Pro	92.1	92.1	92.5	89.7	91	92.3	90.9	89.2	88.8	90.2	1.58
Ala	88.7	85	87.4	82	88.2	85.3	86.5	81.6	86.9	82.8	4.80
Trp	93.8	96.9	94.9	93.7	97.4	94.8	97.8	90	96	91.6	3.96

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Table 5. Variation of immobilized digestive enzyme assay predicted lysine digestibility in soybean meal from different world areas

Amino acid	Asia n = 34		LATAM[1] n = 43		NA[1] n = 233		EU[1] n = 28	
	Mean ± SD(%)	Range (%)	Mean ± SD(%)	Range (%)	Mean ± SD(%)	Range (%)	Mean ± SD(%)	Range (%)
Lys	87.5 ± 4.98	74.3–95.0	89.7 ± 2.25	84.6–93.1	89.6 ± 3.90	70.6–95.5	89.0 ± 3.24	76.8–92.4
Met	85.7 ± 9.66	56.0–98.5	89.7 ± 3.28	82.7–94.5	90.6 ± 5.34	64.1–99.6	90.4 ± 4.63	73.0–95.2
Cys	84.3 ± 8.03	61.2–97.0	87.7 ± 3.52	80.7–94.4	89.2 ± 6.11	59.7–99.0	90.7 ± 5.45	70.2–96.4
Thr	85.5 ± 6.33	66.9–93.7	88.3 ± 2.33	83.7–91.6	89.2 ± 4.34	66.8–94.9	88.9 ± 3.89	74.3–93.0
Arg	89.6 ± 4.30	77.7–95.4	92.3 ± 1.77	88.5–95.1	92.6 ± 3.20	76.7–97.4	92.2 ± 2.72	82.0–95.1
Val	84.0 ± 7.43	62.8–94.1	88.7 ± 3.01	82.3–93.4	89.2 ± 5.50	61.9–97.4	88.7 ± 4.70	71.0–93.6
Ile	85.9 ± 7.39	64.6–95.9	90.5 ± 2.89	84.5–94.9	91.1 ± 5.34	64.6–98.8	91.4 ± 4.68	73.4–97.0
Leu	86.6 ± 6.35	68.5–95.3	90.6 ± 2.59	85.0–94.6	91.0 ± 4.78	67.3–98.0	90.6 ± 4.10	75.1–94.8
His	88.0 ± 5.08	73.3–94.8	91.1 ± 1.97	87.1–94.2	91.6 ± 3.58	73.9–96.9	91.2 ± 3.06	79.7–94.4
Tyr	87.5 ± 8.66	61.9–99.1	92.1 ± 3.08	85.0–97.1	93.9 ± 5.02	69.9–100.0	94.2 ± 4.27	78.1–98.6
Phe	89.1 ± 5.57	74.5–97.5	92.3 ± 2.53	86.6–96.2	92.4 ± 4.54	69.8–99.0	91.8 ± 3.85	77.3–95.8
Asp	85.9 ± 5.17	71.3–92.7	89.2 ± 2.21	84.5–92.8	89.4 ± 3.91	70.1–95.6	89.3 ± 3.74	76.5–98.2
Ser	88.2 ± 6.00	71.6–96.6	92.0 ± 2.58	86.3–96.0	92.4 ± 4.80	68.4–99.1	91.9 ± 4.13	76.3–96.1
Glu	89.9 ± 3.88	79.3–95.1	92.4 ± 1.80	88.5–95.3	92.5 ± 3.14	77.0–97.4	92.0 ± 2.63	82.1–94.8
Pro	89.7 ± 5.41	74.2–97.1	93.0 ± 2.15	88.5–96.3	93.5 ± 4.01	73.5–99.1	93.2 ± 3.46	80.2–96.8
Ala	82.0 ± 6.60	63.0–91.0	86.1 ± 2.58	80.8–90.1	86.7 ± 4.76	63.0–93.6	86.3 ± 4.09	70.9–90.6
Trp	92.5 ± 5.42	75.9–100.0	95.1 ± 3.33	89.0–99.7	95.5 ± 6.22	65.9–100.0	95.6 ± 4.84	76.1–99.0

[1]LATAM = Latin America; NA = North America; EU = European Union.

can be effectively accomplished. These data indicate the broad range in amino acid digestibility predicted for SBM from around the world, indicating the variation that is possible when a single sample is analyzed. However, the mean value difference for each amino acid is within 2 standard deviations of the means for the other world areas. This suggests that the mean values for 46-48% protein SBM amino acid values from over 330 samples obtained from around the world are quite similar. Alternatively, this might indicate that the samples originated in the same country because this was not possible to confirm as indicated above.

In conclusion, we have developed an in vitro IDEA assay for SBM, which has been correlated to poultry TAAD as determined in the cockerel. The IDEA kit assay presented here is a good predictor of SBM TAAD as evidenced by predicting the in vivo true poultry digestibility of a validation set of SBM not included in our standard curve. Furthermore, the power of IDEA as a tool to survey feed ingredients was demonstrated by the analysis of 338 SBM collected from around the world from 2001 to 2005. The IDEA for SBM represents a rapid, robust, and inexpensive predictor of SBM amino acid digestibility compared with in vivo methods. The protein sources that have high variability of amino acid digestibility (e.g., meat and bone meals, poultry by-product meals, and fish meal) emphasize the need for in vitro methodology development.

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