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Prediction equations for estimating nutrient digestibility of feed ingredients based on *in vitro* procedures for pigs

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Competing interests
No potential conflict of interest relevant to this article was reported.

Abstract

The objectives were to assess nutrient digestibility of feed ingredients using *in vitro* procedures and to develop novel prediction equations for estimation of standardized ileal digestibility (SID) of crude protein (CP) and apparent total tract digestibility (ATTD) of gross energy (GE) in feed ingredients for pigs. Ten ingredients (rice, corn, soybean hulls, wheat, wheat bran, palm kernel expellers, copra meal, cashew nuts, rapeseed meal, and soybean meal) were analyzed for GE and CP, ether extract, amylase-treated neutral detergent fiber, acid detergent fiber, and ash concentrations. *In vitro* assays were performed to determine *in vitro* ileal disappearance (IVID) of CP and *in vitro* total tract disappearance (IVTTD) of dry matter in the ingredients. The most suitable equations for feed ingredients fed to pigs were: SID of CP = 16.55 + 0.89 × IVID of CP – 2.00 × ash with R^2 = 0.89; ATTD of GE = 42.68 + 0.57 × IVTTD of dry matter – 2.27 × ash with R^2 = 0.94 (all variables are based on % as-is). In conclusion, the novel prediction equations using *in vitro* nutrient disappearance and ash concentrations can estimate *in vivo* digestibility of protein and energy in feed ingredients for pigs.

Keywords: Feed ingredients, *In vitro* disappearance, Prediction equation, Swine

INTRODUCTION

As the prices of corn, wheat, and soybean meal have risen with fluctuations [1,2], alternative sources have gained increasing attention as a practical solution to reduce feed costs and reliance on traditional feed ingredients [1]. Because nutrient concentrations, including protein and fiber, vary among the alternative feed ingredients, an accurate evaluation of digestibility of nutrients in alternatives is necessary to replace the conventional sources in swine diets [3].

The determination of nutrient digestibility requires animal experiments which are expensive, laborious, and time-intensive. In contrast, *in vitro* procedures mimicking the digestive and absorptive systems in the gastrointestinal tract of pigs to estimate nutrient digestibility in feed ingredients are cost-effective and time-saving [4–7]. *In vitro* procedures have been used for evaluating feed ingredients in previous studies [8,9]. However, *in vivo* nutrient digestibility of feed ingredients may not be accurately estimated solely by *in vitro* disappearance due to the differences in nutrient composition affecting digestion under *in vivo* and *in vitro* conditions [10]. The addition of nutrient concentrations as independent variables may increase the prediction accuracy for nutrient digestibility in pigs. However, to our knowledge, most prediction equations estimating *in vivo* nutrient digestibility were based on *in vitro* disappearance as the sole independent variable [5,11]. For that reason, the objectives of this study were to assess nutrient digestibility of various feed ingredients using *in vitro* procedures and to develop prediction equations for estimating standardized ileal digestibility (SID) of crude protein (CP) and apparent total tract digestibility (ATTD) of gross energy (GE) using *in vitro* nutrient disappearance and nutrient composition in conventional and alternative feed ingredients fed to pigs.

MATERIALS AND METHODS

Test ingredients

Ten feed ingredients, including rice, corn, soybean hulls, wheat, wheat bran, palm kernel expellers, copra meal, cashew nuts, rapeseed meal, and soybean meal, were employed to develop prediction equations for estimation of *in vivo* nutrient digestibility using *in vitro* nutrient disappearance and nutrient concentrations (Table 1). The 10 feed ingredients were chosen to provide a variety of CP and fiber concentrations.

Data collection for *in vivo* digestibility

The values for SID of CP in rice, wheat, and cashew nuts and the values for ATTD of GE in rice, corn, soybean hulls, wheat, wheat bran, palm kernel expellers, copra meal, cashew nuts, and rapeseed meal were from in-house data (Table 2). A total of 44 data for SID of CP in corn, soybean hulls, wheat bran, palm kernel expellers, copra meal, rapeseed meal, and soybean meal were collected from 21 published papers [12–32]. A total of 19 data for ATTD of GE in soybean meal were collected from 9 published papers [8,11,12,15,16,23,30,31,33]. In the search conducted on PubMed and Google Scholar, the keywords included SID of CP, ATTD of GE, feed ingredients, and pigs. The research articles were manually selected according to the

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

Item	Rice	Corn	Soybean hulls	Wheat	Wheat bran	Palm kernel expellers	Copra meal	Cashew nuts	Rapeseed meal	Soybean meal
Dry matter (%)	87.0	86.8	88.4	90.5	86.6	93.4	89.7	95.1	89.6	87.8
Gross energy (kcal/kg)	3,610	3,754	3,779	3,890	4,085	4,505	3,933	6,524	4,281	4,173
Crude protein (%)	8.2	8.3	10.2	12.9	15.2	16.3	21.8	22.4	34.5	48.5
Ether extract (%)	0.3	2.8	2.6	3.3	4.8	9.2	2.1	47.8	2.3	1.3
Ash (%)	0.8	1.3	4.2	2.7	3.8	4.0	6.5	2.6	8.4	6.4
aNDF (%)	1.0	8.1	63.0	9.2	32.8	64.7	58.2	9.1	33.5	5.5
ADF (%)	0.0	2.3	45.4	2.6	10.7	47.4	35.0	4.6	21.6	3.6

aNDF, amylase-treated neutral detergent fiber; ADF, acid detergent fiber.

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Kim BG.
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Formal analysis: Pham TKT.
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Ethics approval and consent to participate
This article does not require IRB/IACUC approval because there are no human and animal participants.

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Table 2. Standardized ileal digestibility (SID) of crude protein (CP) and apparent total tract digestibility (ATTD) of gross energy (GE) in feed ingredients fed to growing pigs

Item	Number of data[1)]	Average digestibility (%)
SID of CP		
Rice	–	96.0
Corn	9	78.5
Soybean hulls	3	62.3
Wheat	–	89.4
Wheat bran	2	70.7
Palm kernel expellers	4	74.5
Copra meal	3	75.8
Cashew nuts	–	86.1
Rapeseed meal	11	72.8
Soybean meal	12	89.2
ATTD of GE		
Rice	–	96.4
Corn	–	88.2
Soybean hulls	–	58.2
Wheat	–	86.8
Wheat bran	–	67.3
Palm kernel expellers	–	64.5
Copra meal	–	67.7
Cashew nuts	–	88.7
Rapeseed meal	–	64.9
Soybean meal	19	87.4

[1)]A total of 44 data for SID of CP in corn, soybean hulls, wheat bran, palm kernel expellers, copra meal, rapeseed meal, and soybean meal fed to growing pigs were obtained from 21 research papers and a total of 19 data for ATTD of GE in soybean meal fed to growing pigs were obtained from 9 research papers [8,11–33]. The values for SID of CP in rice, wheat, and cashew nuts, as well as the values for ATTD of GE in rice, corn, soybean hulls, wheat, wheat bran, palm kernel expellers, copra meal, cashew nuts, and rapeseed meal were determined by our research group as marked with hyphens.

title and nutrient composition of the test ingredients, including CP, fat, fiber, and ash. During the process of screening, data of sows and weaned piglets were removed. The averaged values were calculated and used for each ingredient when multiple data were available for an ingredient.

In vitro ileal disappearance assays (2-step procedure)

In vitro ileal disappearance (IVID) of dry matter (DM) and CP in 10 feed ingredients was evaluated by mimicking the digestive system of pigs based on the procedure developed in the literature [6,34]. The particle size of test ingredients was reduced to less than 1.0 mm using a grinder before the *in vitro* assays. In step 1, one gram of each ingredient was placed into a flask with a capacity of 200 mL, followed by supplementation with 25 mL of 0.1 *M* sodium phosphate buffer solution (pH 6.0) and 10 mL of 0.2 *M* HCl (pH 0.7). To mimic the pigs' stomach digestion, HCl (1 *M*) and NaOH (1 *M*) were poured into the flask to make pH 2.0.

Then, 1.0 mL of 10-mg/mL pepsin solution (P7000, Sigma-Aldrich) was poured into the flask. To prevent fermentation by microorganism, 0.5 mL of chloramphenicol solution (5.0 g/L ethanol; C0378, Sigma-Aldrich) was added. Then, the flasks were agitated using a shaking incubator (LSI-3016R, Daihan Labtech, Namyangju, Korea) at 39℃ for 6 hours.

The next step was performed to mimic the small intestine digestive system of pigs. Initially, 10 mL of 0.2 *M* phosphate buffer solution (pH 6.8) and 5 mL of 0.6 *M* NaOH solution were added to each flask. Next, HCl (1.0 *M*) and NaOH (1.0 *M*) were poured into the flasks to adjust the pH to 6.8, followed by the addition of 1.0 mL of 50-mg/mL pancreatin solution (P1750, Sigma-Aldrich). The flasks were agitated using the shaking incubator at 39℃ for 18 hours. Then, 5 mL of sulfosalicylic acid solution (20%) was added to each flask, and the flasks were kept at room temperature for 30 minutes to precipitate undigested protein. After precipitation for 30 minutes, the undigested samples were filtered using glass filter crucibles (CFE Por. 2; ROBU® Glasfilter-Geraete GmbH, Hattert, Germany), which had been pre-weighed and had contained 0.5 g of celite (Celite 545, Daejung Chemicals & Metals, Siheung, Korea). Then, 10 mL of 95% ethanol was poured twice to wash glass filter crucibles containing undigested samples, followed by 99.5% acetone washing twice. After that, undigested samples in the crucibles were dried at 80℃ for 24 hours. After being cooled in a desiccator for 1 hour, IVID of DM in the test ingredients was calculated by weighing the crucibles. The undigested residues in the filter crucibles were collected for CP analysis to calculate the IVID of CP. The blank values were obtained to correct the contents of DM and CP in the undigested residues not derived from feed ingredient samples, based on the prediction equations using amounts of pepsin and pancreatin added [4]. Three replicates were conducted for each ingredient.

In vitro total tract disappearance assays (3-step procedure)

In vitro total tract disappearance (IVTTD) of DM and organic matter (OM) in 10 test feed ingredients was evaluated by mimicking the digestive system of pigs based on the procedure developed in the literature [5,34]. In steps 1 and 2, only sample weight, enzymes concentration, and incubation period were altered from 2-step *in vitro* assays. For evaluating the IVTTD of DM and OM in feed ingredients, 0.5 g of each sample was digested with pepsin solution (25 mg/mL) in step 1 and pancreatic solution (100 mg/mL) in step 2 with the incubation periods of 2 and 4 hours in step 1 and 2, respectively. In step 3 for mimicking the condition of large intestine fermentation, 10 mL of 0.2 *M* EDTA solution was added to each flask. Then, 30% acetic acid or 1 *M* NaOH was added to adjust the pH to 4.8. The samples in the flasks were supplemented with 0.5 mL of a cellulolytic enzyme mixture (Viscozyme® L, Sigma-Aldrich) and agitated in the incubator at 39℃ for 18 hours. The samples were filtered using glass filter crucibles as suggested in IVID assays, and the residues in the crucibles were dried at 130℃ for 6 hours. Additionally, the ash content in the residues was measured for calculating the IVTTD of OM in test ingredients. A blank was included during the IVTTD procedure to correct the contents of residual DM and OM, which were considered to derive from exogenous enzymes. Three replicates were conducted for each ingredient.

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Chemical analysis

Prior to chemical analyses, the particle sizes of test ingredient samples were reduced by grinding to pass through a 1.0-mm screen. DM (method 930.15), CP (method 990.03), OM (method 942.05), ether extract (EE; method 920.39), ash (method 942.05), amylase-treated neutral detergent fiber (aNDF; method 2002.04), and acid detergent fiber (method 973.18) in feed ingredients were determined according to the AOAC [35]. Gross energy contents in test ingredients were also determined using a bomb calorimeter (6400 Automatic Isoperibol Calorimeter, Parr Instrument Company, Moline, IL, USA).

Calculations

The calculations of *in vitro* DM disappearance followed the equations below [7]:

$$\text{IVID or IVTTD of DM (\%)} = (DM_{ing} - DM_{residues} + DM_{blank}) \div DM_{ing} \times 100 \tag{1}$$

where DM_{ing} (g) is the quantity of DM in feed ingredients, $DM_{residues}$ (g) is the quantity of DM residues after the IVID or IVTTD procedure, and DM_{blank} (g) is the quantity of DM residues in the blank estimated using an estimation model for the 2-step *in vitro* digestion procedure [4]. The quantity of DM residues in the blank after the IVTTD procedure was measured. The calculation of IVID of CP followed the equation below [7]:

$$\text{IVID of CP (\%)} = [(DM_{ing} \times CP_{ing}) - (DM_{residues} \times CP_{residues}) + (DM_{blank} \times CP_{blank})] \div (DM_{ing} \times CP_{ing}) \times 100 \tag{2}$$

where CP_{ing}, $CP_{residues}$, and CP_{blank} are the CP contents in a test ingredient, the undigested residues, and the blank value estimated using the exogenous enzyme doses according to the literature [4], respectively. The calculation of IVTTD of OM followed the equation below [7]:

$$\text{IVTTD of OM (\%)} = (OM_{ing} - OM_{residues} + OM_{blank}) \div OM_{ing} \times 100 \tag{3}$$

where OM_{ing} (g) is the quantity of OM in feed ingredients, $OM_{residues}$ (g) is the quantity of OM in the undigested residues after the IVTTD procedure, and OM_{blank} (g) is the quantity of OM in the blank after the IVTTD procedure.

Statistical analysis

The GLM procedure of SAS (SAS Inst., Cary, NC, USA) was used for data analyses. The fixed effect was test ingredient and least-square means were calculated for IVID and IVTTD of nutrients. The comparison of mean values was made with Tukey's adjustment using the PDIFF option. The experimental unit was a flask. The CORR procedure of SAS was used for correlation analyses among nutrient compositions, *in vitro* disappearance, SID of CP, and energy digestibility. The REG procedure of SAS was used to develop novel prediction equations estimating SID of CP or ATTD of GE based on IVID of CP, IVTTD of DM, nutrient compositions, or both as independent variables. The statistical significance and tendency levels were declared at $p < 0.05$ and $p < 0.10$.

RESULTS

In the 10 feed ingredients, the concentrations of CP ranged from 8.2% to 48.5%, EE ranged from 0.3% to 47.8%, aNDF ranged from 1.0% to 64.7%, ADF ranged from 0% to 47.4%, and ash ranged from 0.8% to 8.4% on an as-is basis (Table 1). The range of GE contents in test ingredients was from 3,610 to 6,524 kcal/kg. The average SID of CP and ATTD of GE in the 10 test ingredients ranged from 62.3% to 96.0% and from 58.2% to 96.4%, respectively (Table 2).

The IVID of DM in wheat was the greatest ($p < 0.05$), followed by rice, corn, and cashew nuts (Table 3). The IVID of CP in cashew nuts and soybean meal was the greatest ($p < 0.05$), followed by rice, wheat, and rapeseed meal. Soybean hulls showed the lowest IVID of DM and CP ($p < 0.05$). The IVTTD of DM in rice was the greatest ($p < 0.05$), followed by cashew nuts, soybean meal, wheat, and corn. The IVTTD of OM in rice, cashew nuts, and soybean meal were the greatest ($p < 0.05$), followed by corn, wheat, and rapeseed meal. Palm kernel expellers showed the lowest IVTTD of DM and OM ($p < 0.05$).

The EE was positively correlated with GE ($r = 0.97$; $p < 0.001$; Table 4). The SID of CP showed a negative correlation with aNDF ($r = –0.81$; $p < 0.01$). The SID of CP was positively correlated with IVID of CP ($r = 0.82$; $p < 0.01$). The ATTD of GE was negatively correlated with aNDF ($r = –0.92$; $p < 0.001$). The ATTD of GE was positively correlated with IVTTD of DM ($r = 0.89$; $p < 0.001$).

The most suitable model for SID of CP was: SID of CP (%) = 16.55 + 0.89 × IVID of CP − 2.00 × ash with $R^2 = 0.89$, and $p < 0.001$ (Table 5). The most suitable model for ATTD of GE was: ATTD of GE (%) = 42.68 + 0.57 × IVTTD of DM − 2.27 × ash with $R^2 = 0.94$, and $p < 0.001$ (Table 6).

Table 3. ***In vitro*** **ileal and total tract disappearance of nutrients in feed ingredients fed to pigs**

Item (%)	Rice	Corn	Soybean hulls	Wheat	Wheat bran	Palm kernel expellers	Copra meal	Cashew nuts	Rapeseed meal	Soybean meal	SEM	*p*-value
In vitro ileal disappearance												
Dry matter	81.7[b]	81.6[b]	17.9[h]	87.4[a]	56.9[e]	32.8[g]	48.6[f]	81.1[b]	61.8[d]	75.9[c]	0.4	< 0.001
Crude protein	88.4[b]	72.6[f]	59.7[g]	85.0[c]	72.8[f]	75.8[e]	79.7[d]	91.4[a]	85.9[c]	90.4[ab]	0.5	< 0.001
In vitro total tract disappearance												
Dry matter	96.7[a]	84.9[d]	49.2[h]	88.1[c]	65.8[g]	43.5[i]	70.9[f]	94.6[b]	77.6[e]	94.5[b]	0.4	< 0.001
Organic matter	96.6[a]	84.8[b]	47.3[f]	87.8[b]	64.6[e]	43.0[g]	69.4[d]	94.4[a]	77.9[c]	93.9[a]	0.6	< 0.001

Each least squares mean represents 3 observations.
[a–i]Least squares of means within a row without a common superscript letter are different ($p < 0.05$).
SEM, standard error of the means.

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Table 4. Correlation coefficients among nutrient concentrations, *in vitro* nutrient disappearance, standardized ileal digestibility (SID) of crude protein (CP), and apparent total tract digestibility (ATTD) of gross energy (GE) in feed ingredients fed to pigs (n = 10)

Item	CP	EE	Ash	aNDF	ADF	IVID of CP	IVTTD of DM	SID of CP	ATTD of GE
GE	0.24	0.97***	−0.01	−0.13	−0.10	0.42	0.18	0.13	0.14
CP		0.04	0.75*	−0.12	−0.09	0.52	0.27	0.14	−0.01
EE			−0.21	−0.17	−0.15	0.34	0.21	0.15	0.22
Ash				0.44	0.42	0.08	−0.24	−0.40	−0.59
aNDF					0.98***	−0.65*	−0.93***	−0.81**	−0.92***
ADF						−0.61	−0.91***	−0.75*	−0.87**
IVID of CP							0.78**	0.82**	0.66*
IVTTD of DM								0.83**	0.89***
SID of CP									0.92***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
EE, ether extract; aNDF, amylase-treated neutral detergent fiber; ADF, acid detergent fiber; DM, dry matter; IVID, *in vitro* ileal disappearance; IVTTD, *in vitro* total tract disappearance.

Table 5. Prediction equations for standardized ileal digestibility (%) of crude protein (CP) based on *in vitro* ileal disappearance (IVID, %) of CP and nutrient concentration (% as-is basis) in pigs (n = 10)

Item	Regression coefficient parameter				Statistical parameter		
	Intercept	IVID of CP	Ash	aNDF	RMSE	R^2	*p*-value
Equation 1	11.59	0.85	–	–	6.28	0.67	0.004
Standard error	16.85	0.21	–	–	–	–	–
p-value	0.511	0.004	–	–	–	–	–
Equation 2	42.95	0.53	–	−0.19	5.19	0.81	0.003
Standard error	20.02	0.23	–	0.09	–	–	–
p-value	0.069	0.054	–	0.066	–	–	–
Equation 3	16.55	0.89	−2.00	–	3.88	0.89	< 0.001
Standard error	10.49	0.13	0.53	–	–	–	–
p-value	0.159	< 0.001	0.007	–	–	–	–

aNDF, amylase-treated neutral detergent fiber; RMSE, root mean square of error.

DISCUSSION

In the present study, the analyzed GE, CP, EE, fiber, and ash contents in the test ingredients were consistent with the values found in the previous studies [8,11–13,15,36]. Among the evaluated ingredients, cashew nuts contained the highest GE content because of the greater EE concentration compared to the other test ingredients [37].

The IVID of CP values in soybean hulls, wheat bran, copra meal, and soybean meal determined in the present study were within the range of data reported in the previous research [1,34,38]. The greater IVID of CP in cashew nuts, soybean meal, and rice compared with the other test ingredients is likely because of the relatively low fiber concentration in cashew nuts, soybean meal, and rice [39,40]. Fibers are more difficult to digest compared to starch, protein,

Table 6. Prediction equations for apparent total tract digestibility (%) of gross energy based on *in vitro* total tract disappearance (IVTTD, %) of dry matter (DM) and nutrient concentration (% as-is basis) in pigs (n = 10)

Item	Regression coefficient parameter				Statistical parameter		
	Intercept	IVTTD of DM	Ash	aNDF	RMSE	R^2	*p*-value
Equation 1	28.29	0.64	–	–	6.74	0.78	0.001
Standard error	9.30	0.12	–	–	–	–	–
p-value	0.016	0.001	–	–	–	–	–
Equation 2	76.69	0.15	–	−0.39	5.87	0.86	0.001
Standard error	26.98	0.28	–	0.21	–	–	–
p-value	0.025	0.608	–	0.102	–	–	–
Equation 3	42.68	0.57	−2.27	–	3.89	0.94	< 0.001
Standard error	6.41	0.07	0.55	–	–	–	–
p-value	< 0.001	< 0.001	0.005	–	–	–	–

aNDF, amylase-treated neutral detergent fiber; RMSE, root mean square of error.

and lipids because pigs excrete fewer endogenous fiber-degrading enzymes [41]. Additionally, fibers in feed ingredients negatively affect the digestibility of other nutrients surrounded by or bonded to fiber fractions, hindering nutrient breakdown and absorption [39,40]. In the *in vitro* condition, exogenous enzymes may be hindered by fiber fractions, resulting in a decrease in *in vitro* disappearance [1]. By the same token, rice showed the greatest IVTTD of DM and OM, and palm kernel expellers showed the lowest IVTTD of DM and OM among the test ingredients mainly because of the fiber concentrations. The values for IVTTD of DM and OM in corn, soybean hulls, wheat, wheat bran, palm kernel expellers, copra meal, rapeseed meal, and soybean meal determined in the present study were within the range of data reported in the previous studies [1,2,11,34,42–44].

The positive relation of GE and EE observed in this work may be a result of the high energy content of lipids [37]. This study found a negative relation between aNDF and SID of CP or ATTD of GE, supporting that fiber is not digested by pigs and disrupts the digestion of other nutrients in feed ingredients, which is confirmed by previous studies [1,45,46]. The positive correlations between IVID of CP and SID of CP, as well as IVTTD of DM and ATTD of GE observed in the present study are in agreement with previous studies that reported relations between *in vitro* disappearance and *in vivo* digestibility, indicating that *in vitro* assays can be adopted to predict SID of CP or ATTD of GE in pigs [5,6,8].

Incorporating aNDF as an additional independent variable in addition to IVID of CP in prediction equations for SID of CP improved the prediction accuracy as the SID of CP was highly correlated with aNDF concentration in feed ingredients. With the same token, the inclusion of aNDF in the prediction model for ATTD of GE increased the determination coefficient as ATTD of GE was highly correlated with aNDF in feed ingredients. However, considering the strong negative correlation between aNDF and SID of CP (r = −0.81, p < 0.01) or ATTD of GE (r = −0.92, p < 0.001), the increased determination coefficients by the inclusion of aNDF in the models for SID of CP and ATTD of GE were not that large (ΔR^2 = 0.14

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and 0.08, respectively). The reason for the lack of large influence of aNDF on the accuracy of prediction models is likely due to the high correlation between aNDF and *in vitro* disappearance, indicating that the effects of aNDF on *in vivo* digestibility may already have been reflected when *in vitro* assays were conducted.

Incorporating ash as an independent variable in addition to *in vitro* nutrient disappearance for estimating SID of CP or ATTD of GE enhanced the prediction accuracy represented by determination coefficients (ΔR^2 = 0.22 and 0.16, respectively) compared with the prediction equations with *in vitro* nutrient disappearance as a sole independent variable. Interestingly, ash content was not correlated with SID of CP and ATTD of GE. The reason for the improved prediction accuracy by the inclusion of ash as an additional independent variable remains unclear. However, previous studies reported that ash contents are negatively correlated with *in vivo* CP digestibility, the amount of digestible CP, and energy utilization in feed ingredients fed to pigs, likely because ash may interfere with nutrient digestion [3,8,47]. Similarly, ash contents in the ingredients tended to be negatively correlated with ATTD of GE (r = −0.59, p = 0.074) in the present study. However, the correlation between ash contents and SID of CP (r = −0.40, p = 0.254) was not significant which may be due to the lack of sufficient number of ingredients or sufficiently large range of ash contents or both in the present study. In addition, the effects of ash on *in vivo* digestibility of energy and nutrients may not be fully reflected under *in vitro* conditions. Further research is warranted to investigate the role and mechanisms of ash in the prediction models for energy and nutrient digestibility.

In conclusion, SID of CP and ATTD of energy in feed ingredients fed to growing pigs can be estimated using prediction models developed in the present study based on *in vitro* nutrient disappearance and ash concentrations as independent variables.

REFERENCES

1. Kim J, Jo YY, Kim BG. Energy concentrations and nutrient digestibility of high-fiber ingredients for pigs based on in vitro and in vivo assays. Anim Feed Sci Technol. 2022;294: 115507. https://doi.org/10.1016/j.anifeedsci.2022.115507
2. Jo H, Sung JY, Kim BG. Effects of supplemental xylanase on in vitro disappearance of dry matter in feed ingredients for swine. Rev Colomb Cienc Pecu. 2021;34:316-23. https://doi.org/10.17533/udea.rccp.v34n4a08
3. Lee SA, Ahn JY, Kim BG. Digestible and metabolizable energy concentrations in cereal grains and byproduct ingredients fed to growing pigs. Anim Feed Sci Technol. 2022;292: 115408. https://doi.org/10.1016/j.anifeedsci.2022.115408
4. Song YS, Kim H, Kim BG. Estimation of dry matter and crude protein residues originating from exogenous digestive enzymes based on the amounts of pepsin and pancreatin used in 2-step in vitro digestibility procedures for non-ruminants. Anim Feed Sci Technol. 2024; 309:115895. https://doi.org/10.1016/j.anifeedsci.2024.115895
5. Boisen S, Fernández JA. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim Feed Sci Technol. 1997;68:277-86. https://doi.org/10.1016/S0377-8401(97)00058-8
6. Boisen S, Fernández JA. Prediction of the apparent ileal digestibility of protein and amino

acids in feedstuffs and feed mixtures for pigs by in vitro analyses. Anim Feed Sci Technol. 1995;51:29-43. https://doi.org/10.1016/0377-8401(94)00686-4
7. Oh J, Kim H, Park K, Kim BG. Drying methods for black soldier fly (Hermetia illucens) larvae as a feed ingredient for pigs affect in vitro nutrient disappearance. Agriculture. 2024;14:1792. https://doi.org/10.3390/agriculture14101792
8. Son AR, Park CS, Kim BG. Determination and prediction of digestible and metabolizable energy concentrations in byproduct feed ingredients fed to growing pigs. Asian-Australas J Anim Sci. 2017;30:546-53. https://doi.org/10.5713/ajas.16.0607
9. Regmi PR, Ferguson NS, Zijlstra RT. In vitro digestibility techniques to predict apparent total tract energy digestibility of wheat in grower pigs. J Anim Sci. 2009;87:3620-9. https://doi.org/10.2527/jas.2008-1739
10. Gandarillas M, Valenzuela MI, Molina J, Arias R, Keim J. In vivo digestibility and in vitro fermentation of high dietary fiber forages in growing pigs' diets. Fermentation. 2023;9:448. https://doi.org/10.3390/fermentation9050448
11. Son J, Park N, Kim BG. Energy utilization of corn, oilseed meals, and fibrous ingredients can be predicted by multi-sample simultaneous in vitroassay for growing pigs. Anim Feed Sci Technol. 2024;309:115903. https://doi.org/10.1016/j.anifeedsci.2024.115903
12. National Research Council. Nutrient requirements of swine. 11th ed. Washington, DC: The National Academies Press; 2012.
13. Li P, Wang F, Wu F, Wang J, Liu L, Lai C. Chemical composition, energy and amino acid digestibility in double-low rapeseed meal fed to growing pigs. J Anim Sci Biotechnol. 2015;6:37. https://doi.org/10.1186/s40104-015-0033-0
14. Traylor SL, Cromwell GL, Lindemann MD, Knabe DA. Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing pigs. J Anim Sci. 2001;79:2634-42. https://doi.org/10.2527/2001.79102634x
15. Sulabo RC, Ju WS, Stein HH. Amino acid digestibility and concentration of digestible and metabolizable energy in copra meal, palm kernel expellers, and palm kernel meal fed to growing pigs. J Anim Sci. 2013;91:1391-9. https://doi.org/10.2527/jas.2012-5281
16. Stein HH, Casas GA, Abelilla JJ, Liu Y, Sulabo RC. Nutritional value of high fiber co-products from the copra, palm kernel, and rice industries in diets fed to pigs. J Anim Sci Biotechnol. 2015;6:56. https://doi.org/10.1186/s40104-015-0056-6
17. Son AR, Hyun Y, Htoo JK, Kim BG. Amino acid digestibility in copra expellers and palm kernel expellers by growing pigs. Anim Feed Sci Technol. 2014;187:91-7. https://doi.org/10.1016/j.anifeedsci.2013.09.015
18. Sauvant D, Perez JM, Tran G. Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish. 2nd ed. Wageningen: Wageningen Academic Publishers; 2004.
19. Rostagno HS, Albino LFT, Calderano AA, Hannas MI, Sakomura NK, Perazzo FG, et al. Brazilian tables for poultry and swine: composition of foods and nutritional requirements. Viçosa: UFV; 2024.
20. Rojas OJ, Stein HH. Effects of reducing the particle size of corn grain on the concentration of digestible and metabolizable energy and on the digestibility of energy and nutrients in corn grain fed to growing pigs. Livest Sci. 2015;181:187-93. https://doi.org/10.1016/j.livsci.2015.09.013
21. Rodriguez DA, Lee SA, de Godoy MRC, Stein HH. Extrusion of soybean hulls does not increase digestibility of amino acids or concentrations of digestible and metabolizable energy

when fed to growing pigs. Transl Anim Sci. 2020;4:txaa169. https://doi.org/10.1093/tas/txaa169

22. Pedersen C, Boersma MG, Stein HH. Energy and nutrient digestibility in NutriDense corn and other cereal grains fed to growing pigs. J Anim Sci. 2007;85:2473-83. https://doi.org/10.2527/jas.2006-620
23. Oliveira MSF, Wiltafsky MK, Lee SA, Kwon WB, Stein HH. Concentrations of digestible and metabolizable energy and amino acid digestibility by growing pigs may be reduced by autoclaving soybean meal. Anim Feed Sci Technol. 2020;269:114621. https://doi.org/10.1016/j.anifeedsci.2020.114621
24. Liu Y, Song M, Almeida FN, Tilton SL, Cecava MJ, Stein HH. Energy concentration and amino acid digestibility in corn and corn coproducts from the wet-milling industry fed to growing pigs. J Anim Sci. 2014;92:4557-65. https://doi.org/10.2527/jas.2014-6747
25. Lee SA, Ahn JY, Son AR, Kim BG. Standardized ileal digestibility of amino acids in cereal grains and co-products in growing pigs. Asian-Australas J Anim Sci. 2020;33:1148-55. https://doi.org/10.5713/ajas.19.0449
26. Kong C, Kang HG, Kim BG, Kim KH. Ileal digestibility of amino acids in meat meal and soybean meal fed to growing pigs. Asian-Australas J Anim Sci. 2014;27:990-5. https://doi.org/10.5713/ajas.2014.14217
27. González-Vega JC, Kim BG, Htoo JK, Lemme A, Stein HH. Amino acid digestibility in heated soybean meal fed to growing pigs. J Anim Sci. 2011;89:3617-25. https://doi.org/10.2527/jas.2010-3465
28. Eklund M, Caine WR, Sauer WC, Huang GS, Diebold G, Schollenberger M, et al. True and standardized ileal digestibilities and specific ileal endogenous recoveries of crude protein and amino acid in soybean meal, rapeseed meal and peas fed to growing pigs. Livest Sci. 2012;145:174-82. https://doi.org/10.1016/j.livsci.2012.01.016
29. Bohlke RA, Thaler RC, Stein HH. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. J Anim Sci. 2005;83:2396-403. https://doi.org/10.2527/2005.83102396x
30. Berrocoso JD, Rojas OJ, Liu Y, Shoulders J, González-Vega JC, Stein HH. Energy concentration and amino acid digestibility in high-protein canola meal, conventional canola meal, and soybean meal fed to growing pigs. J Anim Sci. 2015;93:2208-17. https://doi.org/10.2527/jas.2014-8528
31. Baker KM, Stein HH. Amino acid digestibility and concentration of digestible and metabolizable energy in soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to growing pigs. J Anim Sci. 2009;87:2282-90. https://doi.org/10.2527/jas.2008-1414
32. Almeida FN, Petersen GI, Stein HH. Digestibility of amino acids in corn, corn coproducts, and bakery meal fed to growing pigs. J Anim Sci. 2011;89:4109-15. https://doi.org/10.2527/jas.2011-4143
33. Yang P, Zhao JB, Zhang G, Ni JJ, Huang CF. Determination and prediction of digestible and metabolizable energy of soybean meal and wheat bran for finishing pigs. Livest Sci. 2021;254:104741. https://doi.org/10.1016/j.livsci.2021.104741
34. Son J, Kim BG. Nutrient digestibility of soybean meal products based on in vitroprocedures for pigs. Agriculture. 2023;13:1631. https://doi.org/10.3390/agriculture13081631
35. AOAC. Official methods of analysis. 21st ed. Gaithersburg, MD: Association of Official Analytical Chemists International; 2019.

36. Aletor O, Agbede JO, Adeyeye SA, Aletor VA. Chemical and physio-chemical characterization of the flours and oils from whole and rejected cashew nuts cultivated in southwest Nigeria. Pak J Nutr. 2007;6:89-93. https://doi.org/10.3923/pjn.2007.89.93
37. Whittemore CT, Green DM. Growth of the young weaned pig. The weaner pig: nutrition and management. In: Proceedings of a British Society of Animal Science Occasional Meeting; 2001; Wallingford, UK.
38. Ha DU, Choi H, Kim BG. Supplemental protease improves in vitro disappearance of dry matter and crude protein in feather meal and copra meal for pigs. Rev Bras Zootec. 2020; 49:e20200095. https://doi.org/10.37496/rbz4920200095
39. Navarro DMDL, Abelilla JJ, Stein HH. Structures and characteristics of carbohydrates in diets fed to pigs: a review. J Anim Sci Biotechnol. 2019;10:39. https://doi.org/10.1186/s40104-019-0345-6
40. Bach Knudsen KE. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim Feed Sci Technol. 1997;67:319-38. https://doi.org/10.1016/S0377-8401(97)00009-6
41. Just A, Jørgensen H, Fernández JA. Prediction of metabolizable energy for pigs on the basis of crude nutrients in the feeds. Livest Prod Sci. 1984;11:105-28. https://doi.org/10.1016/0301-6226(84)90012-5
42. Kong C, Park CS, Kim BG. Effects of an enzyme complex on in vitro dry matter digestibility of feed ingredients for pigs. SpringerPlus. 2015;4:261. https://doi.org/10.1186/s40064-015-1060-1
43. Park CS, Son J, Kim BG. Effects of supplemental β-mannanase on in vitro disappearance of dry matter in feed ingredients for swine. Anim Ind Technol. 2022;9:35-40. https://doi.org/10.5187/ait.2022.9.1.35
44. Park KR, Park CS, Kim BG. An enzyme complex increases in vitro dry matter digestibility of corn and wheat in pigs. SpringerPlus. 2016;5:598. https://doi.org/10.1186/s40064-016-2194-5
45. Son AR, Park CS, Park KR, Kim BG. Amino acid digestibility in plant protein sources fed to growing pigs. Asian-Australas J Anim Sci. 2019;32:1745-52. https://doi.org/10.5713/ajas.19.0037
46. Zhang W, Li D, Liu L, Zang J, Duan Q, Yang W, et al. The effects of dietary fiber level on nutrient digestibility in growing pigs. J Anim Sci Biotechnol. 2013;4:17. https://doi.org/10.1186/2049-1891-4-17
47. Noblet J, Perez JM. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. J Anim Sci. 1993;71:3389-98. https://doi.org/10.2527/1993.71123389x