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Original Research Article

An automatically progressed computer-controlled simulated digestion system to predict digestible and metabolizable energy of unconventional plant protein meals for growing pigs

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ABSTRACT

The objective of this experiment was to develop a new computer-controlled simulated digestion system to predict the digestible energy (DE) and metabolizable energy (ME) of unconventional plant protein meals for growing pigs. Nine meals tested included 1 source of rapeseed meal, 4 sources of cottonseed meal, 2 sources of sunflower meal, and 2 sources of peanut meal. Twenty growing pigs (Duroc × [Landrace × Large White]) with an initial body weight (BW) of 41.7 ± 2.6 kg were allotted to a replicated 10 × 3 incomplete Latin square design to determine the DE and ME of 1 basal diet and 9 experimental diets formulated with 9 unconventional plant protein meals. The DE and ME values of unconventional plant protein meals were calculated by the difference method. The in vitro digestible energy (IVDE) of 1 basal diet, 9 experimental diets, and 9 unconventional plant protein meals were determined with 5 replicates of each sample in a complete randomized arrangement. The IVDE/DE or IVDE/ME ranged from 0.96 to 0.98 or 1.00 to 1.01, and the correlation coefficient between IVDE and DE or ME was 0.97 or 0.98 in 10 experimental diets. Accordingly, the IVDE/DE or IVDE/ME ranged from 0.86 to 1.05 or 0.96 to 1.20, and the correlation coefficient between IVDE and DE or ME was 0.92 or 0.91 in 9 unconventional plant protein meals. The coefficient of variation (CV) of IVDE was less than that of DE and ME in the experimental diets (0.43%, 0.80%, and 0.97% for CV of IVDE, DE and ME, respectively) and unconventional plant protein meals (0.92%, 4.84%, and 6.33% for CV of IVDE, DE and ME, respectively). The regression equations to predict DE from IVDE in 10 experimental diets and 9 unconventional plant protein meals were DE = 0.8851 × IVDE +539 (R^2 = 0.9411, residual standard deviation [RSD] = 23 kcal/kg DM, $P < 0.01$) and DE = 0.9880 × IVDE + 166 (R^2 = 0.8428, RSD = 182 kcal/kg DM, $P < 0.01$), respectively. There was no statistical difference in the slopes ($P = 0.82$) or intercepts ($P = 1.00$) of these 2 equations. Thus, 10 diets and 9 unconventional plant protein meals were pooled to establish the regression equation of DE on IVDE as: DE = 0.9813 × IVDE +187 (R^2 = 0.9120, RSD = 118 kcal/kg DM, $P < 0.01$). The regression equations to predict ME from IVDE in 10 experimental diets and 9 unconventional plant protein meals were ME = 0.9559 × IVDE +146 (R^2 = 0.9697, RSD = 18 kcal/kg DM, $P < 0.01$) and ME = 0.9388 × IVDE + 3 (R^2 = 0.8282, RSD = 182 kcal/kg DM, $P < 0.01$), respectively. There was no statistical difference in slopes ($P = 0.97$) but significant difference between the intercepts ($P = 0.02$) of these 2 equations. Our results indicate IVDE has similar response to the DE but different response to the ME in 10 experimental diets and 9 unconventional plant protein meals. Therefore, IVDE is more suitable to predict DE than ME of diets and unconventional plant protein meals for growing pigs.

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1. Introduction

Unconventional plant protein meals are widely used as a source of dietary protein for growing pigs in China, but energy values are variable (Ma et al., 2019; Mateos et al., 2019). Nutritionists require

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quick methods to accurately predict energy values of unconventional plant protein meals for diet formulation. Many techniques have been proposed to quickly predict energy digestibility of feeds for pigs including sources of digestive enzymes, enzyme activities in simulated digestion fluid, steps of digestion and separation of byproducts for in vitro digestion (Swiech, 2017). In vitro enzymatic digestion procedures described by Boisen and Fernandez (1997) have been used to calculate available energy of feed ingredients for pigs (Sauvant et al., 2004; Boisen, 2007). However, predicted and in vivo energy digestibility differed by 4.4% to 9.7% across protein feed ingredients (Boisen and Fernandez, 1997). Woyengo et al. (2016a) also observed high difference (2.6% to 17.4%) between in vitro dry matter digestibility (IVDMD) and apparent ileal digestibility of energy in 1 soybean meal and 4 canola meals for growing pigs. Huang et al. (2017) reported the IVDMD was 7.6% greater than apparent total tract digestibility (ATTD) of dry matter (DM) in 12 corn distillers dried grains with soluble (DDGS) for growing pigs and reported a weak correlation ($R^2 = 0.02$) when the IVDMD was determined with in vitro gastro-small intestine digestion procedures described by Boisen and Fernandez (1997). These studies indicate a substantial gap exists between in vitro and in vivo digestion for protein feed ingredients. The reasons for this disparity may relate to differences between the activity of enzymes and duration of exposure used for in vitro digestion relative to in vivo digestion. Therefore, the objective of this study was to develop a novel in vitro digestion procedure that matched the passage time of diets for growing pigs described by Gao et al. (2018), and mimicked the compositions of simulated gastric, small intestinal and large intestinal fluid of in vivo gastric (Chiang et al., 2008), jejunum (Hu et al., 2010), and cecal (Dang et al., 2018) fluids of growing pigs to accurately predict the DE or ME of unconventional plant protein meals.

2. Materials and methods

All experimental procedures related to the use of pigs were approved by the animal care and welfare committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China). The code of ethical inspection was IAS 2020–28.

2.1. Feed ingredients and experimental diets

Nine unconventional plant protein meals were tested, including 1 source of rapeseed meal (RSM), 4 sources of cottonseed meal (CSM), 2 sources of sunflower meal (SFM), and 2 sources of peanut meal (PM). Their chemical composition is shown in Table 1. A corn soybean meal basal diet was formulated with a crude protein (CP) of 12.28% (Table 2). Nine experimental diets were formulated with corn, soybean meal and test meals as the sources of energy. The ratios of corn to soybean meal in experimental diets were identical to that in the basal diet. The inclusion rates of tested unconventional plant protein meals in the experimental diets were approximately 20% (range: 18.80% to 22.97%), in consideration of antinutrition factors and calculation errors resulting from low inclusion of test ingredients using the substitution method. The CP concentrations of experimental diets were formulated to range from 15.21% to 20.22% according to the recommendation of formula diets for growing-finishing pigs in China (China feed industry association, 2018). All dietary concentrations of calcium, phosphorus, vitamins and trace elements exceeded NRC (2012) requirements for growing pigs (Table 2).

Table 1
Source and chemical composition of unconventional plant protein meals (air-dry basis).

Sample	DM, %	CP, %	Ash, %	EE, %	ADF, %	GE, kcal/kg
RSM	88.69	37.25	6.40	2.91	15.25	4,221
CSM1	90.27	45.91	6.64	0.53	14.81	4,186
CSM2	89.56	48.34	6.96	2.12	11.83	4,262
CSM3	88.83	52.85	6.73	1.86	10.48	4,209
CSM4	92.79	60.49	7.46	1.09	6.63	4,384
SFM1	90.42	37.76	6.56	1.03	18.60	4,193
SFM2	89.53	30.82	5.84	0.80	25.75	4,168
PM1	89.91	47.89	6.05	0.48	13.95	4,178
PM2	91.74	53.83	6.28	0.22	5.82	4,171

DM = dry matter; CP = crude protein; EE = ether extract; ADF = acid detergent fiber; GE = gross energy; RSM = rapeseed meal; CSM = cottonseed meal; SFM = sunflower meal; PM = peanut meal.

2.2. Experimental design

Digestible energy (DE) and metabolizable energy (ME) were tested in all diets according to the procedures described by Regmi et al. (2008) and Adeola and Kong (2014). Twenty pigs (Duroc × [Landrace × Large White]) with an initial BW of 41.7 ± 2.6 kg were allotted to a replicated 10 × 3 incomplete Latin square design, with 10 diets and 3 consecutive periods of 15 d in each. Two pigs per diet were used in each period for a total of 6 replicates per diet. The IVDMD and in vitro DE (IVDE) of basal diet, 9 experimental diets and 9 unconventional plant protein meals were determined with 5 replicates of 1 digestive tube for each sample in a complete randomized arrangement.

2.3. In vivo DE and ME assay

Twenty barrows were housed individually in metabolism crates. Temperature, lighting, and ventilation in the metabolism room was automatically controlled according to environmental parameters recommended by the NY/T388 1999 (China). Each experimental period consisted of an 8-d adaption including an initial 3-d transition from a common commercial diet to experimental diet, 5-d adaption to experimental diet, followed by 5-d collection of urine by a time-to-time method (Pedersen et al., 2007), and 5- to 7-d collection of feces according to the marker (ferric oxide) method (Adeola and Kong, 2014). Feed was provided at a rate of 4% of body weight daily. Pigs were fed 2 equal wet-mix meals at 08:00 and 16:00 every day with ad libitum access to water. From 08:00 on d 9 to 08:00 on d 13, urine was collected in bucket containing 50 mL of 3 mol/L hydrochloric acid and weighed every day. Twenty percent (wt/wt) of urine was sampled and stored at −20 °C. Feces was collected from first to second appearance of ferric oxide and stored at −20 °C. After the end of the experiment, urine and fecal samples were thawed and mixed within the animal and diet. Feces were crushed, ground through a 0.42-mm screen, and the DM content was determined by drying for 72 h in a 65 °C oven followed by cooling and weighing.

2.4. IVDE assay

The computer-controlled simulated digestion system (CCSDS) used to mimic the digestion of stomach, small intestine and large intestine, as well as clearance of byproducts was described by Zhao et al. (2014; Fig. 1). The reagent kits including simulated digestive fluid and buffer solution for growing pigs (product number: IVDEGP) were provided by Hunan Zhongben Intelligent Technology Development Co., Ltd. The simulated gastric fluid comprised 890 U/mL of pepsin hydrochloric acid solution with pH 2.0 (39 °C), according to the results of Chiang et al. (2008) and Wang et al. (2019).

Table 2
Composition and nutrient contents of basal and experimental diets (air-dry basis, %).

Item	Basal diet	Experimental diet								
		1	2	3	4	5	6	7	8	9
Ingredients										
Corn	84.78	65.28	68.63	68.65	68.67	68.66	65.11	68.60	68.66	68.65
Soybean meal	12.91	9.93	10.44	10.43	10.44	10.44	9.90	10.44	10.44	10.44
RSM		22.95								
CSM1			18.81							
CSM2				18.80						
CSM3					18.80					
CSM4						18.81				
SFM1							22.97			
SFM2								18.81		
PM1									18.80	
PM2										18.82
Limestone	0.92	0.38	0.72	0.70	0.63	0.84	0.81	0.71	0.75	0.74
Dicalcium phosphate	0.86	0.93	0.87	0.89	0.93	0.72	0.68	0.91	0.82	0.82
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Premix[1]	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Determined composition										
DM	88.04	87.86	87.89	87.83	87.65	88.36	88.17	87.81	87.79	87.98
GE, kcal/kg	3,837	3,912	3,877	3,891	3,877	3,910	3,902	3,875	3,875	3,886
CP	12.28	17.80	18.00	18.16	18.94	20.22	17.28	15.21	18.38	19.13

RSM = rapeseed meal; CSM = cottonseed meal; SFM = sunflower meal; PM = peanut meal; DM = dry matter; GE = gross energy; CP = crude protein.
[1] The premix provided the following per kilogram of diets (as-fed basis): vitamin A 6,400 IU, vitamin D_3 2,200 IU, vitamin E 50 mg, vitamin K_3 2 mg, vitamin B_1 2 mg, vitamin B_2 5 mg, vitamin B_{12} 24 μg, calcium D-pantothenate 12 mg, nicotinic acid 20 mg, folic acid 1 mg, biotin 120 μg, Cu (as copper sulfate) 10 mg, Fe (as ferrous sulfate) 110 mg, Zn (as zine sulfate) 40 mg, Mn (as manganese sulfate) 25 mg, Se (as sodium selenite) 0.3 mg, I (as potassium iodide) 0.3 mg.

The gastric buffer contained 80.6 mmol/L of NaCl and 6 mmol/L of KCl, and pH was adjusted to 2.0 at 39 °C using 2 mol/L of HCl according to the composition of gastric fluid of pigs described by Fujita et al. (1980). The concentrated simulated small intestinal fluid was composed of 4,239 U/mL of amylase, 1,323 U/mL of trypsin, and 166 U/mL of chymotrypsin according to the in vivo activities of digestive enzyme reported by Hu et al. (2010) and reduction activities during in vitro digestion reported by Wang et al. (2019). The small intestine buffer solution was prepared with 30 mmol/L of Na_2HPO_4, 170 mmol/L of NaH_2PO_4, 89.9 mmol/L NaCl, 15.0 mmol/L of KCl, 0.48 g/L (800,000 units) of penicillin sodium, and the pH was adjusted to 6.44 at 39 °C using 2 mol/L of

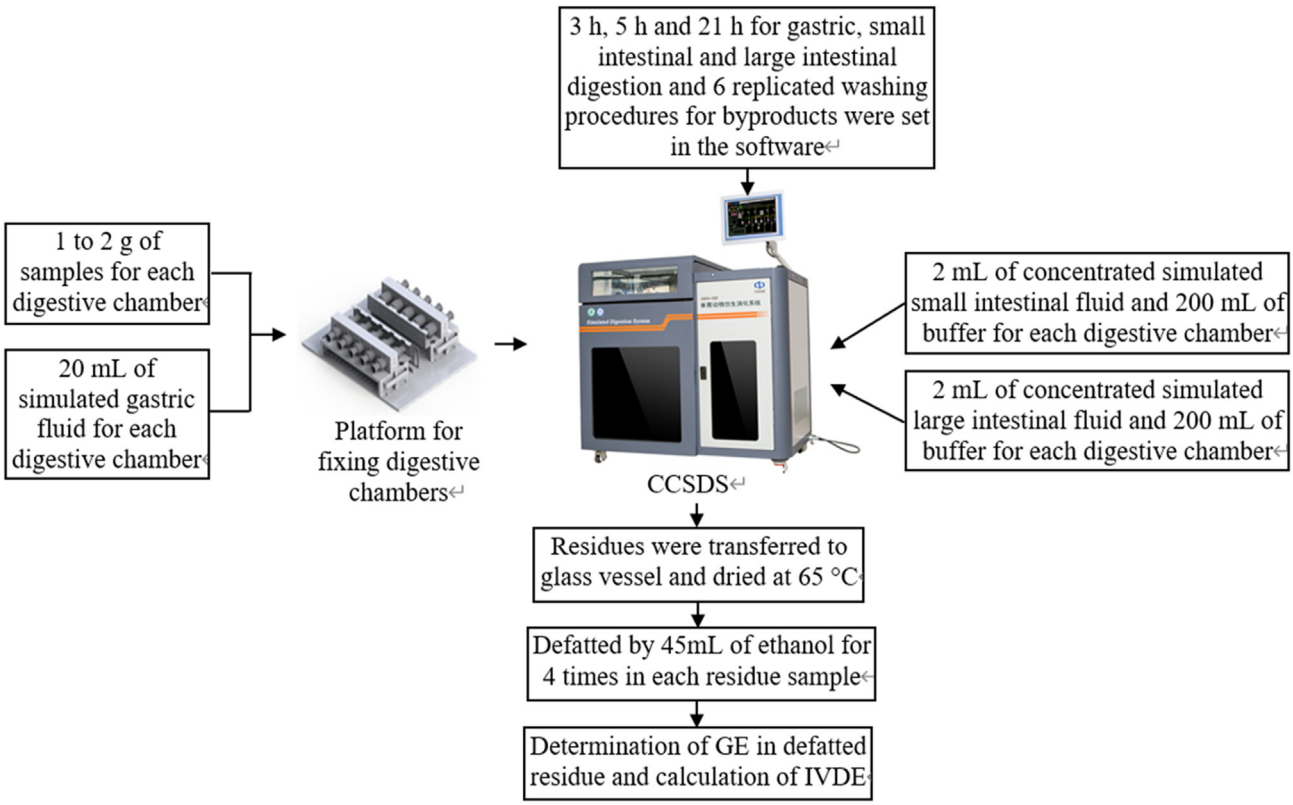

Fig. 1. Schematic diagram of computer-controlled simulated digestion system (CCSDS) to determine in vitro digestible energy (IVDE) of feed for pigs.

NaOH. The concentrated simulated large intestine fluid was composed of 1,572 U/mL of amylase, 491 U/mL of trypsin, 62 U/mL of chymotrypsin, and 0.77 U/mL of cellulase according to the composition of cecal fluid described by Dang et al. (2018). The large intestine buffer was prepared with 30 mmol/L of Na_2HPO_4, 170 mmol/L of NaH_2PO_4, 93.2 mmol/L of NaCl, 11.1 mmol/L of KCl, 0.48 g/L (800,000 units) of penicillin sodium and the pH was adjusted to 6.42 at 39 °C with 2 mol/L of NaOH.

The times for in vitro gastric, small intestinal, and large intestinal digestion were 3, 5 and 21 h, respectively, which matched the passage time of digesta in each segment of digestive tract of growing pigs described by Gao et al. (2018). The digestion incubator was set at 39 °C to match the body temperature of pigs. The in vitro digestion procedure processed by CCSDS: the sample was finely ground through a 0.3-mm sieve (Gao, 2019). Two grams of test diet or 1 g of ingredient sample and 20 mL simulated gastric fluid were added into the digestive chamber of the CCSDS. The gastric buffer was circulated outside the dialysis tubing for 3 h, followed by an emptying procedure of buffer solution. Next, the small intestinal buffer was pumped into the digestive chamber and circulated outside the dialysis tubing for 6 h. When the buffer was circulated for 1 h, 2 mL of concentrated simulated small intestinal fluid was injected into the dialysis tubing of the digestive chamber through the peristaltic pump for simulated small intestinal digestion. After the small intestinal digestion was finished, the emptying procedure of buffer solution automatically progressed. Then, the large intestinal buffer was pumped into the digestive chamber and circulated outside the dialysis tubing for 21 h. When the large intestinal buffer was circulated for 0.1 h, 2 mL of concentrated simulated large intestinal fluid was pumped into the dialysis tubing of the digestive chamber. After the digestion was finished, the buffer was automatically emptied. Six replicated clearance procedures composed of 1,500 mL deionized water circulated outside of dialysis tubing for 4 h followed by an emptying procedure automatically progressed to clear the byproducts. After the simulated digestion, the undigested residue was transferred to a vessel, dried, and defatted in accordance with the description by Zhao et al. (2014).

2.5. Chemical analyses

The DM (method GB/T6435-2006) and gross energy (GE) were analyzed for all samples. The GE was measured using an adiabatic bomb calorimeter (model 6,400; Parr Instrument, Moline, IL, USA) according to the method of ISO 9831:1998. Urine energy was measured as follows: 5 mL of urine was transferred to a crucible with filter paper and dried for 14 h in a 65 °C drying oven. The GE was then measured using an automatic adiabatic bomb calorimeter. The CP (method GB/T6432-1994), ether extract (method 996.01; AOAC, 2007), ash (method 942.15; AOAC, 2007) and ADF (method 973.18; AOAC, 2007) were analyzed for unconventional plant protein meals. The definition of activities of pepsin, amylase, chymotrypsin, trypsin, cellulase were in accordance with the description of Wirnt and Wolf-Peter (1974), Dahlqvist (1962), Wirnt (1974a), Wirnt (1974b) and NY/T912-2004.

2.6. Calculations and statistical analyses

The DE and ME (kcal/kg of DM) in the unconventional plant protein meals was calculated by the difference method according to the mathematical equation described by Adeola and Kong (2014). Outliers were detected as values that deviated between 1.5 times interquartile below the 25th percentile and above the 75th percentile, using the BOXPLOT procedure of SAS (version 9.4; SAS Institute Inc. Cary, NC, USA). The TTSET procedure was performed to compare the in vitro and in vivo values for each of experimental diets and unconventional plant protein meals. Significance was set at $P < 0.05$. The CORR procedure was used to analyze the correlation between IVDE and DE or ME. The REG procedure was used to establish the regression equation of DE or ME on IVDE. The GLM procedure was used to test the difference in slopes and intercepts of linear models of DE or ME against IVDE for experimental diets and unconventional plant protein meals.

3. Results

3.1. The difference between in vitro and in vivo values

The DMD of basal diet and 9 experimental diets was less than the in vivo value (79.88% to 87.69% vs. 82.18% to 89.32%; $P < 0.01$; Table 3). The ratio of in vitro to in vivo DMD of these 10 diets ranged from 0.96 to 0.98. The IVDE or IVDE/GE were less than DE (3,497 to 3,820 kcal/kg of DM vs. 3,623 to 3,923 kcal/kg of DM; $P < 0.01$) or DE/GE (79.25% to 87.65% vs. 82.09% to 89.21%; $P < 0.01$), respectively. The IVDE/DE ranged from 0.96 to 0.98. However, the IVDE or IVDE/GE of 8 in 9 experimental diets was not significantly different from ME (3,497 to 3,792 kcal/kg of DM vs. 3,500 to 3,781 kcal/kg of DM; $P > 0.05$) or ME/GE (79.25% to 85.86% vs.79.31% to 85.60%; $P > 0.05$), respectively. The IVDE/ME in 9 experimental diets ranged from 1.00 to 1.01. The CV of determined IVDE, DE and ME for each of the 10 diets ranged from 0.22% to 0.65%, 0.40% to 1.22% and 0.37% to 1.48%, respectively (Table 5).

The IVDE was less than DE in RSM, CMS2, CSM3, and SFM1 ($P < 0.05$), but the IVDE was greater than DE in CSM2 ($P < 0.01$; Table 4). The IVDE was not statistically different from DE in CSM1, CSM4, SFM2, PM1, and PM2. The IVDE/DE ranged from 0.86 to 1.05. The IVDE/GE was less than DE/GE in RSM, CMS2, CSM3, and SFM1 ($P < 0.05$), but greater than DE/GE in CSM 2 ($P < 0.05$). However, the IVDE/GE was not statistically different from DE/GE in CSM1, CSM4, SFM2, PM1, and PM2. The IVDE was not statistically different from ME in RSM, CSM1, CSM4, SFM2, and PM2, but the IVDE was greater than ME in CSM2, CSM3, SFM1, and PM1 ($P < 0.05$). The IVDE/ME ranged from 0.96 to 1.20. The IVDE/GE was not statistically different from ME/GE in RSM, CSM1, CSM4, SFM2, and PM2, but the IVDE/GE was greater than ME/GE in CSM2 and 3, SFM1, and PM1 ($P < 0.05$). The CV of IVDE, DE and ME for each of the 9 unconventional plant protein meals ranged from 0.51% to 1.91%, 2.58% to 9.05% and 4.25% to 8.62%, respectively (Table 5).

3.2. Regression equations of in vivo values against in vitro values for diets and unconventional plant protein meals

In vitro DMD and in vivo DMD ($r = 0.96$; $P < 0.01$), IVDE and DE ($r = 0.97$; $P < 0.01$), IVDE and ME ($r = 0.98$; $P < 0.01$), IVDE/GE and DE/GE ($r = 0.98$; $P < 0.01$), and IVDE/GE and ME/GE ($r = 0.99$; $P < 0.01$; Table 6) were highly correlated in the 10 diets. In vitro DMD and in vivo DMD ($r = 0.91$; $P < 0.01$), IVDE and DE ($r = 0.92$; $P < 0.01$), IVDE and ME ($r = 0.91$; $P < 0.01$), IVDE/GE and DE/GE ($r = 0.93$; $P < 0.01$) or IVDE/GE and ME/GE ($r = 0.92$; $P < 0.01$; Table 6) were highly correlated in the 9 unconventional plant protein meals. The in vitro and in vivo DMD ($r = 0.97$; $P < 0.01$), IVDE and DE ($r = 0.96$; $P < 0.01$), IVDE and ME ($r = 0.95$; $P < 0.01$), IVDE/GE and DE/GE ($r = 0.97$; $P < 0.01$) and IVDE/GE and ME/GE ($r = 0.97$; $P < 0.01$; Table 6) were highly correlated when data from 10 diets and 9 unconventional plant protein meals were pooled.

The regression equations of DE against IVDE in 10 diets and 9 unconventional plant protein meals were DE = 0.8851 × IVDE +539

Table 3
The DM digestibility and energetic values of 10 diets determined with computer-controlled simulated digestion system and bioassay.

Item	Basal diet	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Digestibility of DM, %										
In vitro	87.69	81.62	82.42	81.81	82.18	84.72	81.00	79.88	83.25	84.43
In vivo	89.32	84.90	84.75	83.60	85.60	86.41	83.38	82.18	85.37	88.35
SEM	0.16	0.44	0.49	0.29	0.23	0.51	0.19	0.41	0.18	0.42
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
In vitro/In vivo	0.98	0.96	0.97	0.98	0.96	0.98	0.97	0.97	0.98	0.96
Available value, kcal/kg										
IVDE	3,820	3,637	3,620	3,602	3,651	3,735	3,569	3,497	3,665	3,792
DE	3,888	3,786	3,746	3,702	3,794	3,846	3,707	3,623	3,763	3,923
ME	3,796	3,636	3,615	3,552	3,655	3,700	3,551	3,500	3,651	3,781
IVDE/DE	0.98	0.96	0.97	0.97	0.96	0.97	0.96	0.97	0.97	0.97
IVDE/ME	1.01	1.00	1.00	1.01	1.00	1.01	1.01	1.00	1.00	1.00
IVDE vs. DE										
SEM	8	20	23	10	11	23	10	21	10	17
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
IVDE vs. ME										
SEM	7	22	14	15	17	26	16	18	19	19
P-value	<0.01	0.96	0.76	<0.05	0.80	0.22	0.28	0.87	0.47	0.56
Efficiency of GE, %										
IVDE/GE	87.65	81.69	82.04	81.32	82.54	84.40	80.63	79.25	83.04	85.86
DE/GE	89.21	85.04	84.92	83.56	85.80	86.92	83.75	82.09	85.26	88.83
ME/GE	87.09	81.66	81.95	80.19	82.64	83.62	80.23	79.31	82.73	85.60
IVDE/GE vs. DE/GE										
SEM	0.19	0.46	0.51	0.22	0.24	0.50	0.23	0.47	0.23	0.37
P-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
IVDE/GE vs. ME/GE										
SEM	0.16	0.49	0.32	0.35	0.39	0.59	0.35	0.41	0.42	0.44
P-value	<0.01	0.96	0.77	<0.05	0.81	0.22	0.28	0.88	0.47	0.56

DM = dry matter; SEM = standard error of the mean; IVDE = in vitro digestible energy; DE = digestible energy; ME = metabolizable energy; GE = gross energy.

Table 4
The energetic values of 9 unconventional plant protein meals determined with computer-controlled simulated digestion system and bioassay.

Item	RSM	CSM1	CSM2	CSM3	CSM4	SFM1	SFM2	PM1	PM2
Available value, kcal/kg									
IVDE	2,974	2,932	3,081	3,310	3,577	2,984	2,461	3,279	3,829
DE	3,448	3,162	2,947	3,438	3,705	3,118	2,524	3,243	3,997
ME	3,107	2,872	2,559	3,097	3,343	2,758	2,274	3,049	3,651
IVDE/DE	0.86	0.93	1.05	0.96	0.97	0.96	0.98	1.01	0.96
IVDE/ME	0.96	1.02	1.20	1.07	1.07	1.08	1.08	1.08	1.05
IVDE vs. DE									
SEM	81	106	38	50	106	45	105	46	80
P-value	<0.01	0.06	<0.01	0.02	0.24	0.01	0.54	0.46	0.07
IVDE vs. ME									
SEM	89	55	72	94	125	72	90	92	95
P-value	0.16	0.28	<0.01	0.04	0.09	<0.01	0.07	0.04	0.09
Efficiency of GE, %									
IVDE/GE	62.62	63.24	64.73	69.85	75.71	64.34	52.76	70.57	83.67
DE/GE	72.46	68.20	61.92	72.54	78.42	67.24	54.12	69.80	87.33
ME/GE	65.28	61.93	53.76	65.37	70.75	59.47	48.77	65.62	79.77
IVDE/GE vs. DE/GE									
SEM	2.25	2.29	0.81	1.06	2.24	0.98	2.25	1.00	1.75
P-value	<0.01	0.06	0.01	0.02	0.24	0.01	0.53	0.46	0.07
IVDE/GE vs. ME/GE									
SEM	2.47	1.20	1.52	1.98	2.64	1.56	1.94	1.99	2.08
P-value	0.32	0.28	<0.01	0.04	0.09	0.01	0.07	0.04	0.09

RSM = rapeseed meal; CSM = cottonseed meal; SFM = sunflower meal; PM = peanut meal; IVDE = in vitro digestible energy; DE = digestible energy; ME = metabolizable energy; SEM = standard error of the mean; GE = gross energy.

(R^2 = 0.9411, RSD = 23 kcal/kg DM, P < 0.01; Fig. 2) and DE = 0.9880 × IVDE + 166 (R^2 = 0.8428, RSD = 182 kcal/kg DM, P < 0.01; Fig. 2), respectively. In these 2 equations, slopes (P = 0.82) and intercepts (P = 1.00) were not statistically different. Consequently, data from 10 diets and 9 unconventional plant protein meals were pooled to establish the regression equation of DE on IVDE as DE = 0.9813 × IVDE +187 (R^2 = 0.9120, RSD = 118 kcal/kg DM, P < 0.01; Fig. 3). The regression equations of ME against IVDE in 10 diets and 9 unconventional plant protein meals were ME = 0.9559 × IVDE +146 (R^2 = 0.9697, RSD = 18 kcal/kg DM, P < 0.01; Fig. 4) and ME = 0.9388 × IVDE +3 (R^2 = 0.8282, RSD = 182 kcal/kg DM, P < 0.01; Fig. 4), respectively. Intercepts were not statistically different (P = 0.02), but slopes differed (P = 0.97) across the equations, so regression equations of ME on IVDE were separated for diets and unconventional plant protein meals.

4. Discussion

In addition to soybean meal, unconventional plant protein meals such as rapeseed meal, cottonseed meal, peanut meal and

Table 5
Coefficient of variation of replicated determination of IVDE, DE or ME for each sample.

Item	Coefficient of variation, %		
	IVDE	DE	ME
Basal diet	0.22	0.44	0.37
Diet 1	0.53	1.10	1.25
Diet 2	0.65	1.22	0.66
Diet 3	0.46	0.40	0.85
Diet 4	0.43	0.49	0.96
Diet 5	0.57	1.20	1.48
Diet 6	0.31	0.56	0.94
Diet 7	0.40	1.21	1.07
Diet 8	0.40	0.50	1.07
Diet 9	0.35	0.88	1.09
Mean	0.43	0.80	0.97
Maximum	0.65	1.22	1.48
Minimum	0.22	0.40	0.37
Unconventional plant protein meals			
RSM	1.03	5.17	6.30
CSM1	0.64	7.42	4.25
CSM2	1.30	2.58	6.13
CSM3	0.53	2.82	5.92
CSM4	0.71	6.29	8.24
SFM1	0.65	2.80	5.11
SFM2	1.91	9.05	8.62
PM1	1.03	3.03	6.64
PM2	0.51	4.43	5.76
Mean	0.92	4.84	6.33
Maximum	1.91	9.05	8.62
Minimum	0.51	2.58	4.25

IVDE = in vitro digestible energy; DE = digestible energy; ME = metabolizable energy; RSM = rapeseed meal; CSM = cottonseed meal; SFM = sunflower meal; PM = peanut meal.

sunflower meal are substantial sources of dietary protein for pigs. The chemical composition and cultivar of oilseed crops contribute to the high variation in the nutritional value of oilseed meals. Bell (1993) summarized 12 studies and concluded that the DE (range from 2,675 to 4,127 kcal/kg of DM) and ME (range from 2,488 to 3,838 kcal/kg of DM) of rapeseed meal for growing pigs mainly depended on the content of glucosinolate and the dehulling rate of rapeseed. Zhang et al. (2012) reported the concentrations of NDF and GE affected the DE of rapeseed meal for growing pigs. In the current study, the DE (3,448 kcal/kg of DM) and ME (3,107 kcal/kg of DM) values of rapeseed meal were close to those reported by NRC (2012) and Oliveira et al. (2020). However, the DE and ME values of rapeseed exceeded values reported by Zhong and Adeola (2019), and were less than those reported by Woyengo et al. (2016b). These findings indicate the energy values of rapeseed meal vary depending on source. The DE (from 2,947 to 3,715 kcal/kg of DM) and ME (from 2,559 to 3,343 kcal/kg of DM) of the current 4 cottonseed meals increased with increasing protein concentration. Similarly, Li et al. (2012) reported positive linear relationships between DE or ME and concentrations of CP and EE in cottonseed meal for growing pigs, and Ma et al. (2018) reported that DE increased from 3,152 to 3,702 kcal/kg of DM as the CP concentration of 5 cottonseed meals increased from 460 to 550 g/kg. In the current 2 sunflower meals, the range of DE values was similar to that described by Liu et al. (2016) and Lyu et al. (2019). Relative to SFM2, SFM1 had a greater CP concentration and less fiber concentration. Consequently, the DE and ME values were greater for SFM1 than SFM 2. These findings agree with results reported by Liu et al. (2016), who observed that DE correlated positively with CP and GE concentration, but negatively correlated with fiber concentration in 10 sunflower meals offered to growing pigs (DE ranged from 2,512 to 2,980 kcal/kg of DM). In the current 2 peanut meals, the DE values were similar to ranges described by Li (2014) and Li et al. (2018). Relative to PM2, the CP concentration was less, and fiber concentration was greater for PM1. As a result, the DE and ME values were less for PM1 than PM2. This finding was consistent with the result in study of Li (2014), who reported the DE or ME correlated positively with CP and negatively correlated with NDF in 12 peanut meals offered to growing pigs.

To account for high variation of digestible nutrients in feed ingredients, in vitro digestion was accepted to evaluate the nutritive values of feed for animals. The method described by Boisen and Fernandez (1997) was the most popular tool to predict digestibility of energy and CP in feed ingredients and diets for growing pigs. The procedure of this method employed pepsin,

Table 6
Pearson correlation coefficients between in vitro and in vivo values in diets and unconventional plant protein meals.

Item	In vitro DMD	In vivo DMD	IVDE	DE	ME	IVDE/GE	DE/GE
Ten diets							
In vivo DMD	0.96*						
IVDE	0.97*	0.99*					
DE	0.89*	0.96*	0.97*				
ME	0.94*	0.99*	0.98*	0.98*			
IVDE/GE	0.99*	0.99*	0.99*	0.93*	0.97*		
DE/GE	0.95*	0.99*	0.99*	0.98*	0.99*	0.98*	
ME/GE	0.97*	1.00*	0.97*	0.94*	0.99*	0.99*	0.99*
Nine unconventional plant protein meals							
In vivo DMD	0.91*						
IVDE	1.00*	0.92*					
DE	0.91*	0.99*	0.92*				
ME	0.90*	0.99*	0.91*	0.99*			
IVDE/GE	1.00*	0.93*	0.99*	0.91*	0.91*		
DE/GE	0.92*	1.00*	0.92*	0.99*	0.99*	0.93*	
ME/GE	0.91*	1.00*	0.91*	0.98*	0.99*	0.92*	0.99*
Ten diets and 9 unconventional plant protein meals							
In vivo DMD	0.97*						
IVDE	0.98*	0.95*					
DE	0.93*	0.96*	0.96*				
ME	0.96*	1.00*	0.95*	0.98*			
IVDE/GE	1.00*	0.97*	0.98*	0.94*	0.97*		
DE/GE	0.97*	1.00*	0.96*	0.98*	1.00*	0.97*	
ME/GE	0.97*	1.00*	0.93*	0.95*	0.99*	0.97*	0.99*

DMD = dry matter digestibility; IVDE = in vitro digestible energy; DE = digestible energy; ME = metabolizable energy; GE = gross energy. *, $P < 0.05$.

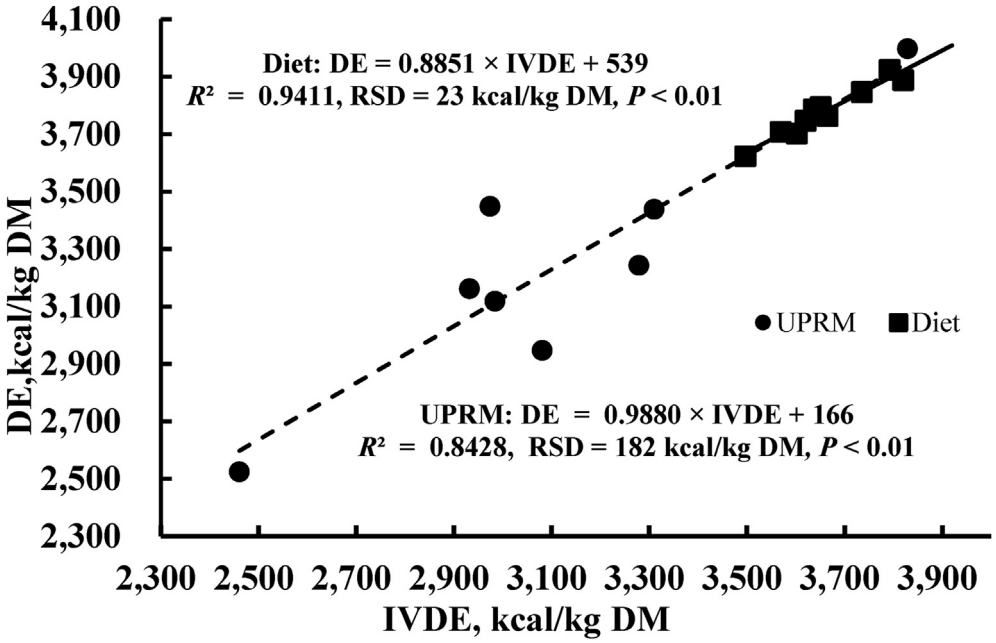

Fig. 2. Linear models to predict digestible energy (DE) from in vitro digestible energy (IVDE) of 10 diets or 9 unconventional plant protein meals (UPRM). Square symbols and solid line and upper equation were for 10 diets. Solid circles and dotted line and below equation were for 9 unconventional plant protein meals. DE value was determined with 6 pigs for each sample and expressed as mean. IVDE value was mean of 5 replicates per sample. RSD = residual standard deviation.

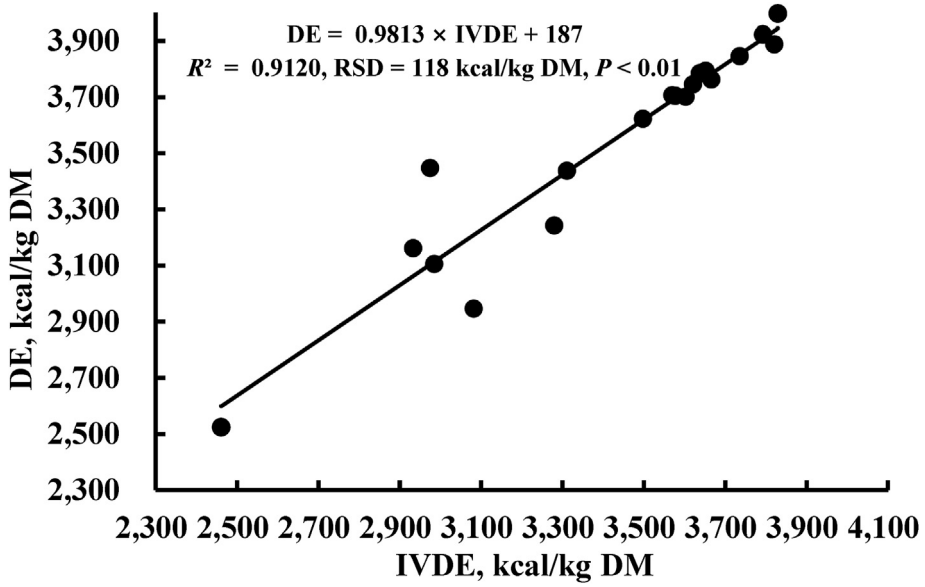

Fig. 3. Linear model to predict digestible energy (DE) from in vitro digestible energy (IVDE) of 10 diets and 9 unconventional plant protein meals. DE value was determined with 6 pigs for each sample and expressed as mean. IVDE value was mean of 5 replicates per sample. RSD = residual standard deviation.

pancreatin and carbohydrates to simulate stomach, small intestine, and large intestine digestion, respectively. After completing in vitro digestion, the undigested residue was separated by filtration, then washed with ethanol and acetone to extract fat. Pujol and Torrallardona (2007) reported the in vitro and in vivo digestibility of organic matter were comparable in 7 barley samples. Noblet and Jaguelin-Peyraud (2007) reported the in vitro and in vivo values were similar and highly correlated (r = 0.91) in 113 diets for growing pigs. However, others report that in vitro values such as digestibility of organic matter (Boisen and Fernandez, 1997) or GE (Sol et al., 2017; Regmi et al., 2008) are generally greater than in vivo energy digestibility for feed ingredients. Inconsistent relationships between in vitro and in vivo values from different samples or studies indicates the reproducibility of various vitro methods is questionable. Across those studies, the concentrations of pepsin for stomach and pancreatin for small intestine all in wt/vol, and the enzymes activities of digestive fluid were unclear. In the current work, we found a small difference in activity of pepsin in pepsin reagents across batches, but great differences in activities of amylase, trypsin and chymotrypsin per gram of pancreatin (CV = 29%; unpublished data in our laboratory) from different batches. Simply using concentration of wt/vol without analyzing

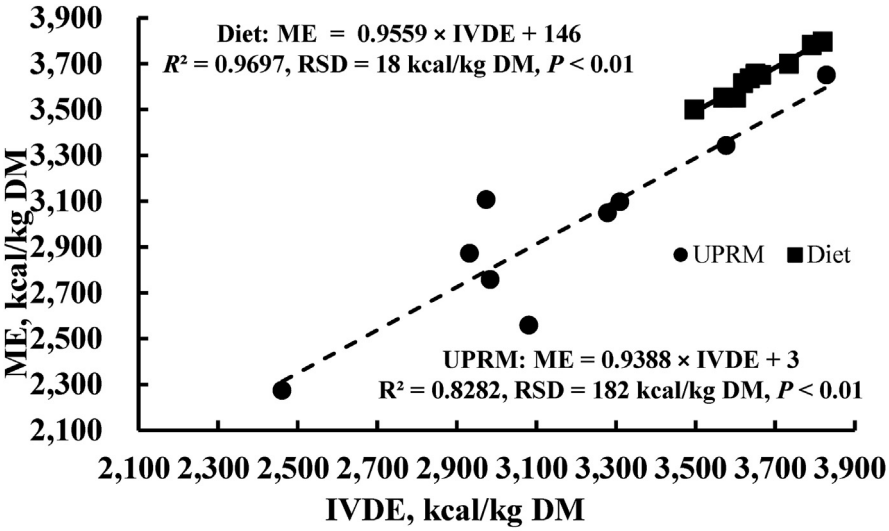

Fig. 4. Linear models to predict metabolizable energy (ME) from in vitro digestible energy (IVDE) of 10 diets or 9 unconventional plant protein meals (UPRM). Square symbols, solid line and upper equation were for 10 diets. Solid circles and dotted line and below equation were for 9 unconventional plant protein meals. ME value was determined with 6 pigs for each sample and expressed as mean. IVDE value was mean of 5 replicates per sample. RSD = residual standard deviation.

enzyme activity may contribute to differences in digestibility and reduced reproducibility of the in vitro method across trials.

In previous in vitro digestion for pigs, only cellulase (Regmi et al., 2008) or complex carbohydrates (Boisen and Fernandez, 1997) were used to simulate digestion of large intestine. However, amylase and proteases are also present in cecal fluid of growing pigs (Dang et al., 2018). Therefore, in vitro digestion of large intestine should be simulated by hydrolysis with proteases and carbohydrates. In recent years, nutritionists have used fresh feces as an inoculum to simulate fermentation of the large intestine in pigs. However, Huang et al. (2018) reported in vitro DM disappearance determined using a gastric-small intestinal simulated digestion following a large intestine fermentation correlated poorly with in vivo GE digestibility of DDGS for pigs. This indicates in vitro fermentation to simulate digestion of large intestine still faces several difficulties, such as the standardization of inoculum, the consistency of in vitro and in vivo fermentation conditions, and the reproductivity of determination (Tao et al., 2019). In contrast, the process simulating in vitro digestion with enzymatic hydrolysis is repeatable by maintaining consistent activities of enzymes in simulated digestive fluid. Brodkorb et al. (2019) established an international in vitro gastrointestinal digestion protocol for the nutritional value of human foodstuffs. In this method, the activity of pepsin, amylase, trypsin and chymotrypsin were evaluated in pepsin and pancreatin, and this information informed development of simulated gastric fluid and simulated small intestinal fluid to match the in vivo activity. In the current study, the in vitro digestion time for stomach, small intestine and large intestine was consistent with the mean passage time of feed in growing pigs (Gao et al., 2018). The activity of pepsin in simulated gastric fluid, activities of amylase, trypsin, and chymotrypsin in small intestinal fluid, and activities of amylase, trypsin, chymotrypsin and cellulase in large intestine fluid were consistent with those of gastric, small intestinal and cecal fluid of growing pigs. Although Pan et al. (2018a; 2018b) used the same type of computer-controlled simulated digestion system described by Zhao et al. (2014) to predict energetic values of corn and sorghum, the duration and digestive enzyme activities of in vitro gastric, small and large intestinal digestion were different from those of growing pigs. Thus, the mean ATTD of GE of 13 corn samples was 9 percentage points above in vitro digestibility of GE (Pan et al., 2018a) and 2 percentage points in 28 sorghum samples (Pan et al., 2018b). This observation indicates that the relationship between in vitro and in vivo values can be inconsistent among different feed ingredients. In the current study, the IVDMD or IVDE was 96% to 98% of the in vivo digestibility of DM or DE, and their correlation coefficient was 0.97 in 10 experimental diets. These results indicate the IVDE measured with the present in vitro digestion were comparable to the DE of experimental diets with unconventional plant protein meals. Similarly, Noblet and Jaguelin-Peyraud (2007) also observed the ratio of in vitro to in vivo organic matter digestibility ranged from 0.99 to 1.04 and their correlation coefficient was 0.94 in 111 mixed diets, in which the in vitro values were determined using the method described by Boisen and Fernandez (1997). This means in vitro enzymatic hydrolysis can accurately predict digestion of dietary organic matter in vivo. In 9 unconventional plant protein meals, IVDE was 86% to 105% of DE with a correlation coefficient of 0.92 and IVDE was 96% to 120% of ME with a correlation coefficient of 0.91. Nine unconventional plant protein meals had a wider range in IVDE/DE or IVDE/ME, which may relate to a greater variation in the 6 replicate determination of DE or ME for each unconventional plant protein meal, caused by the differences in the procedure. In this study, the DE or ME of 6 replicate determination for basal diets differed by 40 kcal/kg of DM and 33 kcal/kg of DM, respectively. The proportion of unconventional plant protein meals in the experimental diet was close to 20%; thus, the range of DE or ME of test ingredient could be calculated as 200 kcal/kg of DM and 165 kcal/kg of DM, respectively. The imprecise DE or ME value of unconventional plant protein meals resulted in greater variation in IVDE/DE or IVDE/ME than that in the diets. This result was supported by that of Noblet and Jaguelin-Peyraud (2007), who observed that the ratio of in vitro to in vivo organic matter digestibility determined with the method of Boisen and Fernandez (1997) ranged from 0.57 to 0.97 in 66 feed ingredients for growing pigs. In this study, the mean CV of IVDE determination was 54% or 44% of that of DE or ME in the experimental diets, and 21% or 17% of that of DE or ME in the unconventional plant protein meals. Consequently, the current simulated digestion procedure was more precise than previously used

bioassay methods, which is in accordance with results described by Zhao et al. (2014). In the regression of DE against IVDE, the model for 10 experimental diets overlapped with that of 9 unconventional plant protein meals, indicating similar relationships between DE and IVDE in diets and unconventional plant protein meals. However, the regression model to predict DE from in vitro organic matter digestibility of 79 mash diets was different from that for 66 feed ingredients when determined with the method of Boisen and Fernandez (1997) as reported by Noblet and Jaguelin-Peyraud (2007). This finding indicates that the current in vitro method has better adaptability than the in vitro method described by Boisen and Fernandez (1997) to estimate DE of diets and feed ingredients for pigs. The linear model of ME against IVDE for 10 experimental diets had the same slopes but different intercepts compared to these of the model for 9 unconventional plant protein meals. Because ME/DE was negatively related to CP content in feeds (NRC, 2012), this ratio was higher in experimental diets than in unconventional plant protein meals (0.96 vs. 0.90). The inconsistent ratio of ME to DE in diets and unconventional plant protein meals indicates differences in the extend of metabolism for pigs. Therefore, IVDE is more suitable to predict DE than ME of diets and feed ingredients for growing pigs.

5. Conclusions

Our study showed that the IVDE determined with a novel, automatically progressed, computer-controlled simulated digestion system was similar to and related predictably to DE and ME of both experimental diets and unconventional plant protein meals. The linear model of DE against IVDE for experimental diets overlapped with that for unconventional plant protein meals, while the linear model of ME against IVDE for experimental diets had the same slopes but different intercepts from that for unconventional plant protein meals. Our findings indicate IVDE is more suitable to predict DE of diets and unconventional plant protein meals than ME for growing pigs.

Author contributions

Feng Zhao designed the research; **Zhongyuan Du**, **Mingqiang Song** and **Shuli Zeng** conducted the research; **Zhongyuan Du** and **Yuming Wang** analyzed the data; **Feng Zhao**, **Zhongyuan Du** and **Yuming Wang** wrote the paper; **Lixiang Gao** and **Jiangtao Zhao** provided financial support, pigs, feedstuffs and metabolic room. **Feng Zhao** had the primary responsibility for final content. **Feng Zhao** and **Zhongyuan Du** decided to submit the paper for publication. All authors read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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References

Adeola O, Kong C. Energy value of distillers dried grains with solubles and oilseed meals for pigs. J Anim Sci 2014;92:164–70.
Association of Official Analysis Chemists (AOAC). Official methods of analysis. 18th ed. MD: AOAC Int. Gaithersberg; 2007.
Bell JM. Factors affecting nutritional value of canola meal: a review. Can J Anim Sci 1993;73:679–97.
Boisen S. A new concept for practical feed evaluation systems. Denmark: Faculty of Agricultural Sciences, University of Aarhus; 2007.
Boisen S, Fernandez 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.
Brodkorb A, Egger L, Alminger M, Alvito P, Assunção R, Ballance S, et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc 2019;14:991–1014.
Chiang CC, Croom J, Chuang ST, Chiou PWS, Yu B. Development of a dynamic system simulating pig gastric digestion. Asian Austral J Anim 2008;21:1522–8.
China feed industry association. Formula feeds for starter and growing-finishing pigs. Beijing: T/CFIAS001-2018); 2018.
Dahlqvist A. A method for the determination of amylase in intestinal content. Scand J Clin Lab Invest 1962;14(2):145–51.
Dang F, Zhao F, Gao L, Gao Q, Wang Y, Zhang H. Effects of dietary protein levels and fiber sources on characteristics of cecal fluid of growing pigs. Chin J Anim Nutr 2018;30:2510–8.
Fujita S, Kokue E, Kurebayashi Y, Hayama T. Secretory kinetics of electrolytes in porcine gastric juice from Heidenhain pouch. Nihon Juigaku Zasshi 1980;42:401–6.
Gao. Study on determination of effective energy in cereal grains based on simulating in vivo digestion process for growing pigs. Chinese Agricultural University; 2019. p. 20–34 [Master Degree Thesis Dissertation].
Gao Q, Zhao F, Zhang H, Wang Y, Liu B. Effects of dietary crude fiber level and feeding time on passage rate of digesta in intestinal tract of growing pigs. Chin J Anim Nutr 2018;30:5230–7.
Hu G, Zhao F, Zhang H, Zhong Y, Liu Z. Effect of the source and level of dietary protein on the comparision of jejunal fluid in growing pigs. Chin J Anim Nutr 2010;22:1220–5.
Huang Z, Urriola PE, Shurson GC. Use of in vitro dry matter digestibility and gas production to predict apparent total tract digestibility of total dietary fiber for growing pigs. J Anim Sci 2017;95:5474–84.
Huang Z, Urriola PE, Shurson GC. Prediction of digestible and metabolizable energy of corn distillers dried grains with solubles for growing pigs using in vitro digestible nutrients. J Anim Sci 2018;96:1818–24.
Li QY. Model of available energy content and amino acid digestibility of peanut meals for growing pigs [Master Degree Thesis Dissertation]. China agricutural university; 2014.
Li JT, Li DF, Zang JJ, Yang WJ, Zhang WJ, Zhang LY. Evaluation of energy digestibility and prediction of digestible and metabolizable energy from chemical composition of different cottonseed meal sources fed to growing pigs. Asian-Australas J Anim Sci 2012;25:1430–8.
Li Y, Li Z, Liu H, Noblet J, Liu L, Li D. Net energy content of rice bran, corn germ meal, corn gluten feed, peanut meal, and sunflower meal in growing pigs. Asian-Australas J Anim Sci 2018;31:1481–90.
Liu J, Xu X, Zhao PF, Tian QY, Zhang S, Li P. Evaluation of energy digestibility and prediction of digestible and metabolisable energy in sunflower seed meal fed to growing pigs. Ital J Anim Sci 2016;14.
Lyu Z, Zang J, Lai C, Li P, Ma D, Zhao J. Effects of dietary fibre level and body weight of pigs on nutrient digestibility and available energy in high-fibre diet based on wheat bran or sunflower meal. J Anim Physiol Anim Nutr 2019;103:1895–907.
Ma DL, Ma XK, Liu L, Zhang S. Chemical composition, energy, and amino acid digestibility in 7 cottonseed co-products fed to growing pigs. J Anim Sci 2018;96:1338–49.
Ma XK, Hu JX, Shang QH, Liu HS, Piao XS. Chemical composition, energy content and amino acid digestibility in cottonseed meals fed to growing pigs. J Appl Anim Res 2019;47:280–8.
Mateos GG, Camara L, Fondevila G, Lazaro RP. Critical review of the procedures used for estimation of the energy content of diets and ingredients in poultry. J Appl Poultry Res 2019;28:506–25.
Noblet J, Jaguelin-Peyraud Y. Prediction of digestibility of organic matter and energy in the growing pig from an in vitro method. Anim Feed Sci Technol 2007;134:211–22.
NRC. Nutrient requirements of swine. 11th rev. Washington, DC: Natl. Acad. Press; 2012.
Oliveira MSF, Wiltafsky-Martin MK, Stein HH. Excessive heating of 00-rapeseed meal reduces not only amino acid digestibility but also metabolizable energy when fed to growing pigs. J Anim Sci 2020;98.
Pan L, Ma H, Piao XS, Liu L, Li DF. A computer-controlled simulated digestion system is a promising in vitro digestibility technique to predict 'digestible energy of corn grain for growing pigs. Anim Feed Sci Technol 2018a;235:43–9.
Pan L, Piao XS, Wu Y, Ma H, Ma XK, Shan QH. Digestible energy of sorghum grain for pigs could be predicted using a computer-controlled simulated digestion system. Anim Feed Sci Technol 2018b;240:31–9.
Pedersen C, Boersma MG, Stein HH. Digestibility of energy and phosphorus in ten samples of distillers dried grains with solubles fed to growing pigs. J Anim Sci 2007;85:1168–76.

Pujol S, Torrallardona D. Evaluation of in vitro methods to estimate the in vivo nutrient digestibility of barley in pigs. Livest Sci 2007;109:186–8.
Regmi PR, Sauer WC, Zijlstra RT. Prediction of in vivo apparent total tract energy digestibility of barley in grower pigs using an in vitro digestibility technique. J Anim Sci 2008;86:2619–26.
Sauvant D, Perez J-M, Tran G. Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish. Wageningen: Wageningen Academic Publishers; 2004.
Sol C, Castillejos L, Lopez-Verge S, Gasa J. Prediction of the digestibility and energy contents of non-conventional by-products for pigs from their chemical composition and in vitro digestibility. Anim Feed Sci Technol 2017;234:237–43.
Swiech E. Alternative prediction methods of protein and energy evaluation of pig feeds. J Anim Sci Biotechnol 2017;8:39.
Tao S, Bai Y, Zhou X, Zhao J, Yang H, Zhang S. In vitro fermentation characteristics for different ratios of soluble to insoluble dietary fiber by fresh fecal microbiota from growing pigs. ACS Omega 2019;4:15158–67.
Wang Y, Zhao F, Zhang H, Dang FK, Gao QT, Yu Y. Study on the variation of digestive enzyme activities depending on digestion time in the simulated digestion for pigs Chinese. J Vet Anim Sci 2019;50:1412–23.
Wirnt R. In: Ul[rich Bergmeyer H, editor. Chymotrypsin: Measurements with N-benzoyl-L-tyrosine ethyl ester as substrate. Methods of enzymatic analysis. 3rd ed., vol. 2. Weinheim, Germany: Verlag Chemie; 1974a. p. 1009–12.
Wirnt R. In: Ulrich Bergmeyer H, editor. Trypsin, measurement with nα-p-toluenesulfonyl-l-arginine methyl ester as substrate. Methods of Enzymatic Analysis. 3rd ed. Weinheim, Germany: Verlag Chemie; 1974b. p. 1021–4.
Wirnt R, Wolf-Peter F. In: Ulrich Bergmeyer H, editor. Pepsin. Methods of enzymatic analysis. 3rd ed. Weinheim, Germany: Verlag Chemie; 1974. p. 1050–2.
Woyengo TA, Jha R, Beltranena E, Zijlstra RT. In vitro digestion and fermentation characteristics of canola co-products simulate their digestion in the pig intestine. Animal 2016a;10:911–8.
Woyengo TA, Sanchez JE, Yanez J, Beltranena E, Cervantes M, Morales. A Nutrient digestibility of canola co-products for grower pigs. Anim Feed Sci Technol 2016b;222:7–16.
Zhang T, Liu L, Piao XS. Predicting the digestible energy of rapeseed meal from its chemical composition in growing-finishing pigs. Asian-Australas J Anim Sci 2012;25:375–81.
Zhao F, Ren LQ, Mi BM, Tan HZ, Zhao JT, Li H. Developing a computer-controlled simulated digestion system to predict the concentration of metabolizable energy of feedstuffs for rooster. J Anim Sci 2014;92:1537–47.
Zhong R, Adeola O. Energy values of solvent-extracted canola meal and expeller-derived canola meal for broiler chickens and growing pigs determined using the regression method 1. J Anim Sci 2019;97:3415–25.