

# Sensitivity of in vitro digestible energy determined with computer-controlled simulated digestion system and its accuracy to predict dietary metabolizable energy for roosters

Y. Yu,<sup>\*</sup> F. Zhao,<sup>\*,1</sup> J. Chen,<sup>\*</sup> Y. Zou,<sup>†</sup> S. L. Zeng,<sup>\*</sup> S. B. Liu,<sup>†</sup> and H. Z. Tan<sup>†</sup>

*\*The State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China; and <sup>†</sup>Wen's Food Group Co. Ltd., Yunfu, Guangdong 527439, China*

**ABSTRACT** Two experiments were conducted to validate the sensitivity and accuracy of in vitro digestible energy (IVDE) determined with a computer-controlled simulated digestion system (CCSDS) to predict metabolizable energy (ME) of diets for roosters. In experiment 1, soybean hulls were added to a basal diet (calibration diet 1) at 2.06, 4.12, 6.17, 8.23, 10.28, 12.32, or 14.37% of the diets (calibration diets 2–8) to produce an interval of approximately 80 kcal ME/kg. The sensitivity was measured by comparing the determined and actual IVDE of the diets. With these data, a linear model was developed to predict ME from IVDE. In experiment 2, validation diets were identical except they were composed of different cereal ingredients. For each diet, the correlations and ratios between IVDE and ME were analyzed to test the sensitivity of IVDE to predict ME across different ingredients. In experiment 1, a slope of 0.9899 was calculated in a linear regression of determined IVDE on actual IVDE ( $R^2 = 0.9998$ ;  $P < 0.01$ ). The ratio of

determined IVDE to actual IVDE was 0.9878. The ratio of IVDE to apparent metabolizable energy (AME) and to nitrogen-corrected AME (AMEn) was 1.03 and 1.05, respectively. The linear models to predict ME from IVDE were  $AME = 0.8449 \times IVDE + 451$  ( $R^2 = 0.9812$ , residual standard deviation [RSD] = 28 kcal/kg;  $P < 0.01$ ) and  $AMEn = 0.8357 \times IVDE + 436$  ( $R^2 = 0.9821$ , RSD = 27 kcal/kg;  $P < 0.01$ ). In experiment 2, a significant simple correlation was observed between the IVDE and AME or AMEn of validation diets ( $r > 0.97$ ;  $P < 0.01$ ). The ratio of IVDE to AME and to AMEn was 1.04 and 1.05, respectively. Predicted and determined AME or AMEn of 8 validation diets differed by less than 100 kcal/kg. The regression of determined AME or AMEn against predicted AME or AMEn ( $R^2 \geq 0.9466$ ;  $P < 0.01$ ) resulted in an overlapped line where  $Y = X$ . These results suggest the IVDE determined with CCSDS is highly sensitive and can be used to accurately predict the ME of diets for roosters across a wide range of cereal grains.

**Key words:** diet, in vitro digestible energy, metabolizable energy, rooster, simulated digestion system

2021 Poultry Science 100:206–214  
<https://doi.org/10.1016/j.psj.2020.09.070>

## INTRODUCTION

Dietary metabolizable energy (ME) strongly influences the cost of feed for poultry, and it is the first consideration for formulation. Under many practical conditions, the ME of commercial diets is calculated from tabulated energetic values of individual ingredients and their concentrations (Mateos et al., 2018) and can be confirmed using in vivo energy balance experiments (Hill and Anderson, 1958; Sibbald, 1976; Farrell, 1978;

Sibbald, 1983; Bourdillon et al., 1990). However, the in vivo procedures are inefficient, time-consuming, expensive, and consequently insufficient to rapidly determine ME values when producing commercial diets (Mateos et al., 2018). Therefore, the development of a rapid, standardized laboratory procedure to estimate the ME of diets is of interest to poultry nutritionists (Jha and Tiwari, 2016; Świąch, 2017; Mateos et al., 2018).

In vitro digestion can predict nutrient digestibility of feed for poultry because it mimics the major process of in vivo digestion (Sakamoto et al., 1980; Clunies et al., 1984; Valdes and Leeson, 1992; Losada et al., 2009, 2010; Yegani et al., 2013; Zhao et al., 2014; Bryan et al., 2018; Pan et al., 2018). However, conventional in vitro digestion has redundant steps that are subject to an operator who must manually adjust the digestion

© 2020 Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received July 3, 2020.

Accepted September 28, 2020.

<sup>1</sup>Corresponding author: [zhaofeng@caas.cn](mailto:zhaofeng@caas.cn)

**Table 1.** Composition and nutrient content of diets for roosters (DM basis).

Items	Calibration diets in experiment 1								Validation diets in experiment 2							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Ingredients, %																
Corn	64.26	62.93	61.61	60.29	58.97	57.65	56.34	55.02	-	-	-	-	25.70	25.70	-	25.70
Wheat	-	-	-	-	-	-	-	-	64.26	-	-	-	38.56	-	-	-
Barley	-	-	-	-	-	-	-	-	-	64.26	-	-	-	38.56	32.13	19.28
Rough rice	-	-	-	-	-	-	-	-	-	-	64.26	-	-	-	-	-
Paddy	-	-	-	-	-	-	-	-	-	-	-	64.26	-	-	32.13	19.28
Soybean meal	20.06	19.64	19.23	18.82	18.40	18.00	17.59	17.18	20.06	20.06	20.06	20.06	20.06	20.06	20.06	20.06
Corn gluten meal	6.29	6.16	6.03	5.90	5.77	5.64	5.51	5.39	6.29	6.29	6.29	6.29	6.29	6.29	6.29	6.29
Soybean oil	5.18	5.07	4.97	4.86	4.76	4.64	4.54	4.43	5.18	5.18	5.18	5.18	5.18	5.18	5.18	5.18
Sodium chloride	0.34	0.33	0.33	0.32	0.31	0.31	0.29	0.29	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Limestone	1.32	1.30	1.26	1.24	1.21	1.18	1.15	1.13	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32
Dicalcium phosphate	1.85	1.82	1.77	1.74	1.70	1.66	1.63	1.59	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85
Lysine-sulphate	0.31	0.30	0.30	0.29	0.29	0.28	0.28	0.26	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
DL-Methionine	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Vitamin-mineral premix <sup>1</sup>	0.22	0.22	0.21	0.21	0.20	0.20	0.20	0.19	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Choline chloride	0.11	0.11	0.11	0.10	0.10	0.10	0.10	0.10	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Soybean hulls	0.00	2.06	4.12	6.17	8.23	10.28	12.32	14.37	-	-	-	-	-	-	-	-
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Nutrient content <sup>2</sup>																
DM, %	91.22	88.55	88.41	89.44	89.91	90.32	89.56	91.07	90.01	90.80	88.77	89.72	88.44	89.40	89.13	89.08
GE, kcal/kg	4,732	4,718	4,658	4,690	4,685	4,620	4,613	4,602	4,668	4,677	4,639	4,668	4,670	4,698	4,679	4,611
CP, %	21.07	20.57	20.49	20.63	20.08	20.18	19.49	19.12	24.39	22.60	20.76	20.30	22.95	21.75	21.83	20.90
Ether extract, %	5.64	5.84	5.64	4.82	4.56	3.39	4.73	3.35	5.11	5.84	5.89	2.75	5.83	5.79	2.81	2.96
Crude fiber, %	2.91	4.19	4.81	5.91	6.51	7.54	7.85	8.76	3.38	5.30	2.51	9.14	2.82	4.34	7.47	5.47
Crude ash, %	5.33	5.38	5.35	5.29	5.31	5.36	5.26	5.21	5.65	6.11	5.27	6.22	5.53	5.84	6.17	5.89

Abbreviation: GE, gross energy.

<sup>1</sup>Supplied per kilogram of diet 1: vitamin A, 5,000 IU; vitamin D3, 1,000 IU; vitamin E, 10.0 IU; vitamin K3, 0.50 mg; thiamine, 1.8 mg; riboflavin, 3.0 mg; vitamin B6, 3.0 mg; vitamin B12, 10.0 µg; pantothenic acid, 10.0 mg; nicotinic acid, 25.0 mg; folic acid, 0.55 mg; biotin, 0.15 mg; Cu (as copper sulfate), 8.0 mg; Fe (as ferrous sulfate), 80 mg; Mn (as manganese sulfate), 80 mg; Zn (as zinc sulfate), 60 mg; I (as calcium iodate), 0.35 mg; Se (as sodium selenite), 0.15 mg.

<sup>2</sup>Values were determined values (DM basis).

**Table 2.** Chemical composition of cereal grains for formulating validation diets in the experiment 2 (DM basis).

Item	Corn	Wheat	Barley	Rough rice	Paddy
DM, %	87.53	89.16	90.14	86.48	89.64
GE, kcal/kg	4,470	4,466	4,403	4,368	4,419
CP, %	8.79	14.86	11.97	8.61	7.95
Ether extract, %	4.34	2.41	2.68	3.11	2.06
Crude fiber, %	2.42	3.04	6.40	1.62	12.34
Crude ash, %	1.42	1.89	2.56	1.59	2.70

Abbreviation: GE, gross energy.

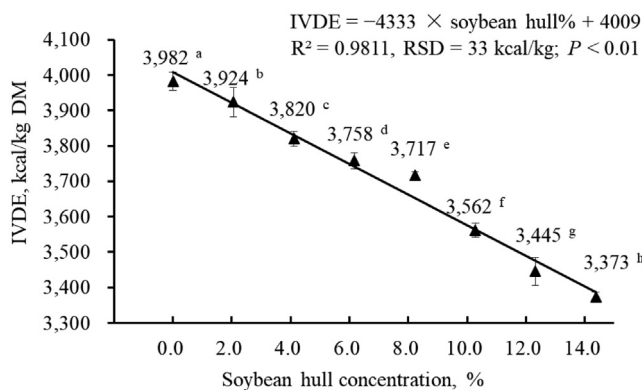
conditions and separate the digested byproduct (Sakamoto et al., 1980; Clunies et al., 1984; Clunies and Leeson, 1984; Valdes and Leeson, 1992; Boisen and Fernández, 1997; Yegani and Korver, 2012). Recently, we developed a novel, computer-controlled simulated digestion system (CCSDS) to automatically predict the digestibility of feed for roosters (Zhao et al., 2014). The in vitro digestible energy (IVDE) determined with CCSDS was highly correlated with ME across 16 feed ingredients (Zhao et al., 2014). However, little work has verified these findings in compound diets. Furthermore, the sensitivity of IVDE is unknown when the dietary ME values vary by 50 to 100 kcal/kg. Using rooster as an animal model, the objectives of this study were to 1) test the sensitivity of IVDE of diets across a wide range of ME and 2) establish and validate a prediction model of ME from IVDE in compound diets made with various cereal grains.

## MATERIALS AND METHODS

All experimental procedures 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 2019-53.

### Experimental Design

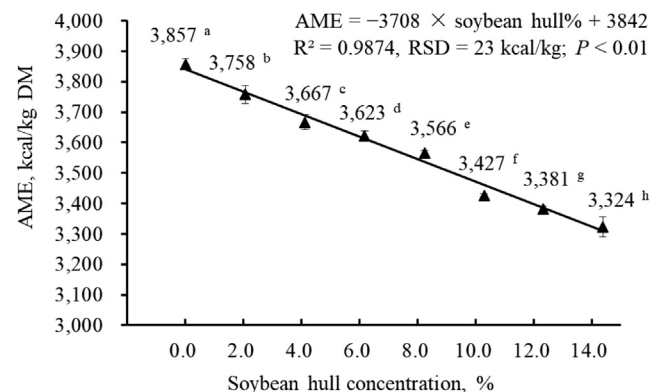
**Experiment 1** The objective of this experiment was to measure the sensitivity of IVDE across diets diluted by



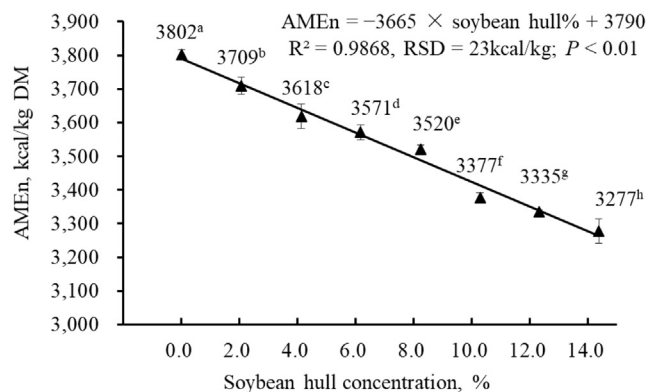
**Figure 1.** Linear model of in vitro digestible energy (IVDE) regressed on soybean hulls concentration in 8 calibration diets. Means with no common superscripts differ significantly ( $P < 0.05$ ). IVDE value was mean of 5 replicates per sample and expressed as mean  $\pm$  SD. Abbreviation: RSD, residual standard deviation.

increasing levels of soybean hulls (Table 1) and establish a prediction model of ME from IVDE. Calibration diet 1 was formulated to exceed the nutrient requirements of yellow-feathered chicken (China Agricultural Industry Standard, 2004). Soybean hulls were added into the basal diet at 2.06, 4.12, 6.17, 8.23, 10.28, 12.32, or 14.37% to produce calibration diets 2 to 8 resulting in an interval of about 80 kcal ME/kg across diets calculated on the tabulated ME value of ingredients in China (Institute of Animal Sciences of CAAS, 2019). The IVDE was determined with CCSDS in 5 replicates, and ME was determined in 4 replicates of 3 yellow-feathered roosters. The sensitivity was measured by comparing the determined and actual IVDE of the diets. With these data, a linear model was developed to predict ME from IVDE.

**Experiment 2** The objective of this experiment was to test the sensitivity of IVDE and accuracy of ME prediction model using 8 validation diets (Table 1). Eight validation diets were formulated to meet or exceed the nutrient requirement of yellow-feathered chicken (China Agricultural Industry Standard, 2004). Validation diets 1 to 8 were identical except contained different compositions of cereal grains (Table 2). Dietary IVDE was assessed in 5 replicates, and dietary ME was assessed in 4 replicates of 3 yellow-feathered roosters. The relationship between dietary IVDE and ME was analyzed to test the sensitivity of IVDE to predict ME. The difference between predicted and determined ME of validation



**Figure 2.** Linear model of AME regressed on soybean hulls concentration in 8 calibration diets. Means with no common superscripts differ significantly ( $P < 0.05$ ). AME value was determined with 12 roosters for each sample and expressed as mean  $\pm$  SD. Abbreviations: AME, apparent metabolizable energy; RSD, residual standard deviation.



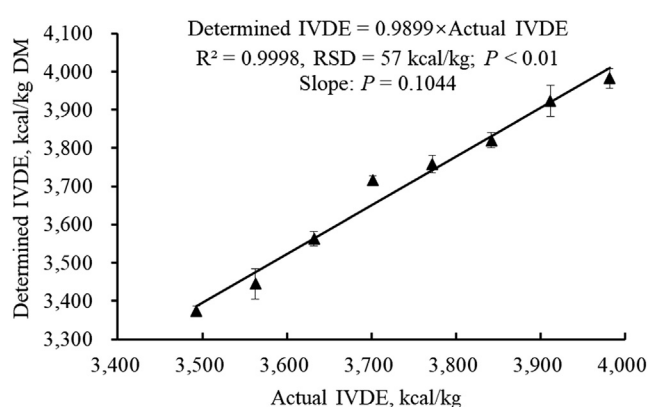
**Figure 3.** Linear model of AMEn regressed on soybean hulls concentration in 8 calibration diets. Means with no common superscripts differ significantly ( $P < 0.05$ ). AMEn value was determined with 12 roosters for each sample and expressed as mean  $\pm$  SD. Abbreviations: AMEn, nitrogen-corrected apparent metabolizable energy; RSD, residual standard deviation.

diets was used to test the accuracy of ME prediction model established in experiment 1.

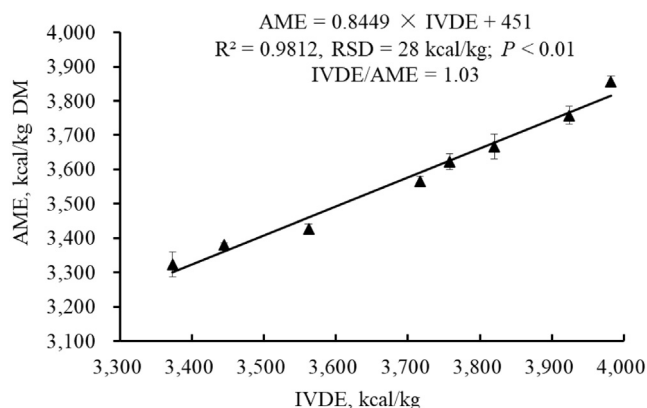
### IVDE Determination

The CCSDS automatically simulated the in vivo digestion processes of gizzard intestine as described by Zhao et al. (2014). Parameters for controlling the mix frequency, digestion temperature, digestion time for gastric or small intestinal phase, liquid waste removal, and wash procedures for byproducts were set in accordance with those described by Zhao et al. (2014).

The gastric buffer solution was composed of 16.9 mmol/L NaCl, 9.6 mmol/L KCl, and 10 mmol/L HCl and adjusted to pH 2.0 at 41°C by the addition of 200 mmol/L HCl to correspond with in vivo digestion conditions of roosters (Sturkie, 1976). The simulated gastric fluid was made of 1,550 U/mL pepsin (Sigma 10,070; Sigma-Aldrich Co., St. Louis, MO). The anterior intestinal buffer solution was prepared with 85.8 mmol/L



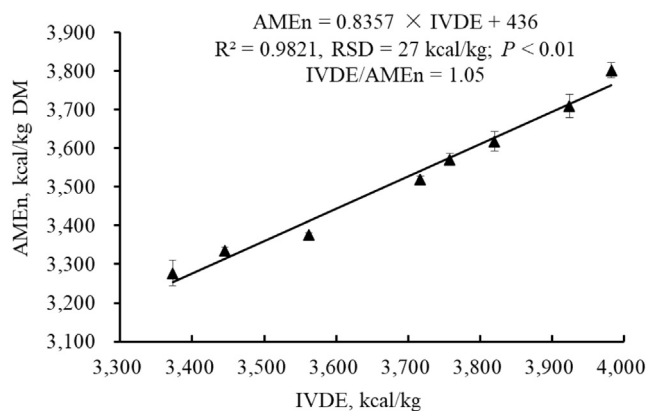
**Figure 4.** Linear model of determined in vitro digestible energy (IVDE) regressed on actual IVDE in 8 calibration diets. Determined IVDE value was mean of 5 replicates per sample. Actual IVDE value was calculated according to the determined IVDE of basal diet or soybean hulls and their concentrations in the calibration diet. Abbreviations: RSD, residual standard deviation;  $H_0$ : slope = 1;  $H_A$ : slope  $\neq$  1.



**Figure 5.** Linear model to predict AME from in vitro digestible energy (IVDE) of 8 calibration diets contained 0, 2.06, 4.12, 6.17, 8.23, 10.28, 12.32, or 14.37% soybean hulls. AME value was determined with 12 roosters for each sample and expressed as mean  $\pm$  SD. IVDE value was mean of 5 replicates per sample. Abbreviations: RSD, residual standard deviation; IVDE/AME, ratio of IVDE to AME.

L NaCl, 18.7 mmol/L KCl, 170 mmol/L  $\text{NaH}_2\text{PO}_4$ , and 30 mmol/L  $\text{Na}_2\text{HPO}_4$  and adjusted to pH 6.50 at 41°C by the addition of 200 mmol/L NaOH. The posterior intestinal buffer solution was composed of 85.8 mmol/L NaCl, 18.7 mmol/L KCl, 30 mmol/L  $\text{NaH}_2\text{PO}_4$ , and 170 mmol/L  $\text{Na}_2\text{HPO}_4$  and adjusted to pH 7.99 at 41°C by the addition of 200 mmol/L NaOH. The concentrated simulated intestinal fluid was composed of 4,416 U/mL amylase (Sigma A3306), 542 U/mL trypsin (A600626; BBI Co. Ltd., Shanghai, China), and 124 U/mL chymotrypsin (Amresco 0164; Amresco Inc., Solon, OH) according to the digestive enzyme activities in the small intestinal fluid of roosters (Zhao et al., 2010).

In brief, 2 g of diet and 20 mL simulated gastric fluid were added into the dialysis tubing of the digestion chamber of the CCSDS. The gastric buffer circulated for 4 h outside of dialysis tubing, then digested product was washed with deionized water to simulate the digestive processes of the gizzard. Subsequently, concentrated



**Figure 6.** Linear model to predict AMEn from in vitro digestible energy (IVDE) of 8 calibration diets contained 0, 2.06, 4.12, 6.17, 8.23, 10.28, 12.32 or 14.37% soybean hulls. AMEn value was determined with 12 roosters for each sample and expressed as mean  $\pm$  SD. IVDE value was mean of 5 replicates per sample. Abbreviations: RSD, residual standard deviation; IVDE/AMEn, ratio of IVDE to AMEn.

**Table 3.** The determined and predicted values for ME in 8 validation diets in the experiment 2.

Validation diets	IVDE <sup>1</sup> , kcal/kg	AME, kcal/kg				IVDE/AME <sup>7</sup>	AMEn, kcal/kg				IVDE/AMEn <sup>7</sup>
		Determined <sup>2</sup>	Predicted <sup>3</sup>	Difference <sup>4</sup>	CI <sup>6</sup>		Determined <sup>2</sup>	Predicted <sup>5</sup>	Difference <sup>4</sup>	CI <sup>6</sup>	
1	3,847 <sup>c</sup>	3,607 <sup>c</sup>	3,701	-94	3,627-3,921	1.07	3,569 <sup>c</sup>	3,651	-82	3,579-3,919	1.08
2	3,513 <sup>g,f</sup>	3,394 <sup>e</sup>	3,419	-25	3,344-3,588	1.04	3,370 <sup>e</sup>	3,372	-2	3,299-3,586	1.04
3	4,096 <sup>a</sup>	3,929 <sup>a</sup>	3,912	17	3,826-4,182	1.04	3,886 <sup>a</sup>	3,859	27	3,776-4,179	1.05
4	3,498 <sup>g</sup>	3,348 <sup>e</sup>	3,406	-58	3,331-3,574	1.04	3,316 <sup>f</sup>	3,359	-43	3,286-3,571	1.05
5	3,899 <sup>b</sup>	3,671 <sup>b</sup>	3,745	-74	3,669-3,975	1.06	3,644 <sup>b</sup>	3,694	-50	3,621-3,972	1.07
6	3,715 <sup>d</sup>	3,570 <sup>c</sup>	3,590	-20	3,518-3,787	1.04	3,533 <sup>c</sup>	3,541	-8	3,471-3,785	1.05
7	3,531 <sup>f</sup>	3,458 <sup>d</sup>	3,434	24	3,360-3,606	1.02	3,406 <sup>e</sup>	3,387	19	3,315-3,603	1.04
8	3,619 <sup>e</sup>	3,505 <sup>d</sup>	3,509	-4	3,436-3,692	1.03	3,466 <sup>d</sup>	3,460	6	3,390-3,689	1.04
Mean	3,715	3,560	3,590	-29		1.04	3,524	3,540	-17		1.05
Minimum	3,498	3,348	3,406	-94		1.02	3,316	3,359	-82		1.04
Maximum	4,096	3,929	3,912	24		1.07	3,886	3,859	27		1.08
Range <sup>8</sup>	598	581	506			0.05	570	500			0.04
SEM	11	20					16				
ANOVA, <i>P</i> value	<0.0001	<0.0001					<0.0001				
<i>r</i>		0.9727					0.9781				
<i>P</i> value		<0.0001					<0.0001				
Estimates of regression <sup>9</sup>											
Intercept <sup>10</sup>		48					36				
<i>P</i> -value		0.8932					0.9085				
Slope <sup>11</sup>		0.9786					0.9851				
<i>P</i> value		0.8286					0.8669				
R <sup>2</sup>		0.9466					0.9568				

<sup>a-g</sup>Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

Abbreviations: AME, apparent metabolizable energy; AMEn, nitrogen-corrected apparent metabolizable energy; IVDE, in vitro digestible energy; ME, metabolizable energy; RSD, residual standard deviation.

<sup>1</sup>IVDE = in vitro digestible energy, mean of 5 determinations per sample.

<sup>2</sup>The value was determined with 12 roosters for each sample.

<sup>3</sup>The value was calculated based on:  $AME = 0.8449 \times IVDE + 451$  ( $R^2 = 0.9812$ ,  $RSD = 28$  kcal/kg;  $P < 0.01$ ).

<sup>4</sup>Difference was calculated as Determined-Predicted.

<sup>5</sup>The value was calculated based on:  $AMEn = 0.8357 \times IVDE + 436$  ( $R^2 = 0.9821$ ,  $RSD = 27$  kcal/kg;  $P < 0.01$ ).

<sup>6</sup>95% confidence intervals for the predicted values of ME.

<sup>7</sup>IVDE/ME = Ratio of IVDE to determined ME.

<sup>8</sup>Range = maximum - minimum.

<sup>9</sup>Regression of determined values on predicted values for ME.

<sup>10</sup>H<sub>0</sub>: intercept = 0; H<sub>a</sub>: intercept  $\neq$  0.

<sup>11</sup>H<sub>0</sub>: slope = 1; H<sub>a</sub>: slope  $\neq$  1.



simulated intestinal fluid was injected into the dialysis tubing by peristalsis pump. Anterior and posterior intestinal buffers continuously circulated for 7.5 h to simulate the digestive processes of the small intestine. After completion of the simulated digestion, the undigested residues were transferred to a preweighed vessel and dried at 65°C overnight followed by 5 h at 105°C. Dry residues were then transferred to a preweighed sintered glass crucible (G4) to extract fat with 45 mL of ethanol 4 times. Finally, defatted residues were dried at 105°C for 5 h to constant weight.

### ***In Vivo ME Assay***

In experiment 1, 96 Chinese yellow-feathered roosters (Wen's Yellow A; BW = 3.48 ± 0.14 kg; 21.5-week-old) were individually weighed and allocated to 8 calibration diets in a randomized complete block design. Roosters were blocked into 4 body weight blocks, and 3 cages represented each of the 8 calibration diets. In experiment 2, another 96 Chinese yellow-feathered roosters (Wen's Yellow A; BW = 3.95 ± 0.17 kg; 24-week-old) were allocated to 1 of 8 validation diets with 4 replicates of 3 roosters for each diet similar to the experiment design of experiment 1. All roosters were individually housed (0.45 × 0.45 × 0.55 m) and provided with free access to water via a suspended nipple drinker line in an environmentally controlled room (23°C) with 16 h of light per day.

The ME bioassay was adopted from methods described by Bourdillon et al. (1990). Roosters were acclimated for 55 h after which the experimental diet was withdrawn for 17 h. Roosters were then allowed free access to experimental diet for 79 h, deprived of feed for 17 h, and excreta was collected for 96 h to determine the apparent metabolizable energy (AME). The nitrogen-corrected apparent metabolizable energy (AMEn) was calculated by AME corrected to zero nitrogen balance according to Hill et al. (1960).

### ***Chemical Analysis***

Samples were ground finely in a laboratory mill fitted with a 0.3-mm mesh screen before chemical analysis. The DM content (method 934.01; AOAC, 1990) was determined by oven drying at 105°C for 5 h. Diet, excreta, and residue samples were analyzed for gross energy (GE) by a Parr 6400 automatic adiabatic calorimeter (Parr Instrument Co., Moline, IL) with benzoic acid as the calibration standard. Diets were analyzed for CP (Kjeldahl N; method 954.01; AOAC, 1990), ether extract (method 920.39; AOAC, 1990), crude fiber (method 962.09; AOAC, 1990), and ash (method 942.05; AOAC, 1990).

### ***Calculation and Statistical Analysis***

The IVDE was calculated using the following formula described by Zhang et al. (2019):  $IVDE = [(sample\ DM\ weight \times sample\ GE) - (defatted\ residue\ DM\ weight \times defatted\ residue\ GE) + GE\ of\ dry\ residue\ of\ digestive\ enzymes] / sample\ DM\ weight$ . The ME was

calculated as follows:  $AME\ (kcal/kg\ of\ DM) = (energy\ intake - energy\ output) / feed\ intake$ .  $AMEn\ (kcal/kg\ of\ DM) = [AME - 8.22 \times (nitrogen\ intake - nitrogen\ output)] / feed\ intake$ .

The mean, SD, and range for IVDE or ME were calculated with the MEANS procedure of SAS 9.0 (SAS Inst. Inc., Cary, NC). The GLM procedure of SAS 9.0 (SAS Inst. Inc., Cary, NC) was used to assess the effect of soybean hulls and diet on IVDE and ME. Duncan's test for multiple comparisons was conducted ( $\alpha = 0.05$ ). The regression of IVDE or ME on soybean hulls concentration was developed in the REG procedure of SAS 9.0 (SAS Inst. Inc., Cary, NC). The determined IVDE was compared to actual IVDE calculated according to the IVDE of basal diet and concentration of soybean hulls to establish sensitivity. A linear regression of determined and actual IVDE and prediction model of ME from IVDE was established using the REG procedure. The ME prediction models were used to predict ME of validation diets, and the relationship between determined and predicted ME was evaluated using the REG procedure. Evaluation of slope (equal to 1) and intercept (equal to 0) was conducted using the TEST selection of REG procedure. The coefficient of determination ( $R^2$ ) and residual standard deviation (RSD) indicated quality of the regression models with a lower RSD indicating a better fit model (Kaps and Lamberson, 2004). If determined and predicted ME differed by less than 100 kcal/kg of DM, it was considered to be acceptable accuracy (Valdes and Leeson, 1992).

## **RESULTS**

### ***Sensitivity of IVDE for Diet and Regression Models of ME on IVDE***

In experiment 1, dietary energy values declined linearly with increasing soybean hulls from 3,982 to 3,373 kcal/kg for IVDE, 3,857 to 3,324 kcal/kg for AME, or 3,802 to 3,277 kcal/kg for AMEn in diets with 0 to 14.37% soybean hulls ( $P < 0.01$ ; Figures 1–3). The regression model of dietary IVDE, AME, and AMEn on soybean hulls concentration was  $IVDE = -4,333 \times \text{soybean hulls } \% + 4,009$  ( $R^2 = 0.9811$ ,  $RSD = 33$  kcal/kg;  $P < 0.01$ ; Figure 1),  $AME = -3,708 \times \text{soybean hulls } \% + 3,842$  ( $R^2 = 0.9874$ ,  $RSD = 23$  kcal/kg;  $P < 0.01$ ; Figure 2) or  $AMEn = -3,665 \times \text{soybean hulls } \% + 3,790$  ( $R^2 = 0.9868$ ,  $RSD = 23$  kcal/kg;  $P < 0.01$ ; Figure 3). Therefore, with each 1% of dietary soybean hulls, the dietary IVDE declined by 43.33 kcal/kg, AME declined by 37.08 kcal/kg, and AMEn declined by 36.65 kcal/kg. The actual value of dietary IVDE could be calculated according to the IVDE values of the basal diet and soybean hulls and their dietary concentrations. The actual IVDE ranged from 3,493 to 3,982 kcal/kg, and determined IVDE ranged from 3,373 to 3,982 kcal/kg across 8 calibration diets. The relationship of determined IVDE with actual IVDE can be expressed as  $IVDE = 0.9899 \times \text{actual IVDE}$  ( $R^2 = 0.9998$ ,  $RSD = 57$  kcal/kg;  $P < 0.01$ ; Figure 4). However, the

slope was not different from 1 ( $P = 0.1044$ ). The ratio of determined IVDE to actual IVDE was 0.9889 (SD = 0.0157). In the 8 calibration diets, the ratio of dietary IVDE to AME or AMEn was 1.0339 (SD = 0.0112) and 1.0483 (SD = 0.0111), respectively. Dietary IVDE, AME, and AMEn values ranged by 609, 533, or 525 kcal/kg across 8 diets. The IVDE and AME or AMEn were correlated linearly ( $r > 0.99$ ,  $P < 0.01$ ; [Figures 5 and 6](#)). The linear models to predict ME from IVDE were  $AME = 0.8449 \times IVDE + 451$  ( $R^2 = 0.9812$ , RSD = 28 kcal/kg;  $P < 0.01$ ; [Figure 5](#)) and  $AMEn = 0.8357 \times IVDE + 436$  ( $R^2 = 0.9821$ , RSD = 27 kcal/kg;  $P < 0.01$ ; [Figure 6](#)).

### Validation for Sensitivity of IVDE and Accuracy of ME Prediction Models

In experiment 2 ([Table 3](#)), validation diet 3 had the greatest IVDE, AME, and AMEn relative to other diets ( $P < 0.05$ ), and diet 5 had the second greatest AME and AMEn. Dietary IVDE and AME were lower for diets 2 and 4 relative to other diets ( $P < 0.05$ ), and AMEn was lowest for validation diet 4 ( $P < 0.05$ ). The IVDE values of validation diet 5, 1, 6, 8, and 7 significantly decreased in sequence ( $P < 0.05$ ). The AME of validation diets 1 and 6 or 7 and 8 did not differ ( $P > 0.05$ ), but AME of validation diets 1 and 6 was greater than that of diets 7 and 8 ( $P < 0.05$ ). Dietary AMEn of validation diets 1 and 6 or 2 and 7 did not differ ( $P > 0.05$ ), but AMEn of validation diets 1 and 6 were greater than those of 2 and 7 ( $P < 0.05$ ). The AMEn of validation diet 8 was less than that of 1 and 6 but greater than that of 2 and 6. Dietary IVDE, AME, or AMEn varied by 598, 581, or 570 kcal/kg, respectively, across 8 validation diets. The ratio of IVDE to AME or AMEn was 1.04 or 1.05. The IVDE was highly correlated with AME or AMEn of the validation diets ( $r > 0.97$ ;  $P < 0.01$ ).

The ME values for 8 validation diets could be predicted by the ME prediction models from IVDE of calibration diets in experiment 1 ([Table 3](#)). Predicted and determined ME differed by less than 100 kcal/kg, which accounts for 2.6 to 3.0% of AME and AMEn. In the regression of determined ME against predicted ME ( $R^2 > 0.94$ ;  $P < 0.01$ ), the slope was not significantly different from 1 (slope = 0.9786,  $P = 0.8286$  for AME; slope = 0.9851,  $P = 0.8669$  for AMEn), and intercept was not significant different from 0 (intercept = 48,  $P = 0.8932$  for AME; intercept = 36,  $P = 0.9085$  for AMEn).

## DISCUSSION

Previous studies randomly selected calibration samples to establish energy prediction equations for swine ([Boisen and Fernández, 1997](#); [Noblet and Jaguelin-Peyraud, 2007](#); [Regmi et al., 2008](#); [Sol et al., 2017](#); [Pan et al., 2018](#)) or poultry ([Wiseman et al., 2000](#); [Zhao et al., 2014](#)). Generally, energy values are normally distributed ([Boisen and Fernández, 1997](#); [Wiseman et al., 2000](#); [Noblet and Jaguelin-Peyraud, 2007](#); [Zhao et al., 2014](#); [Sol et al., 2017](#)), and as a result, extreme

data points can create leverage ([Regmi et al., 2008](#)) and influence regression estimations ([Kaps and Lamberson, 2004](#)). In the use of near-infrared reflectance spectroscopy to predict chemical composition of feeds, calibration samples were selected according to score values of principal component analysis or distance in a cluster analysis for samples scanned by 1,100 to 2,500 nm spectroscopy ([China National Standard, 2002](#)). This method eliminates repeated observations in the calibration samples and the risk of high leverage. In experiment 1, the energetic values of calibration diets declined linearly with increasing dietary levels of soybean hulls. The determined IVDE of soybean hull was 578 kcal/kg, which is similar to values of 480 kcal/kg reported by [Wei et al. \(2019\)](#). Others have reported the AME and AMEn of soybean hulls are 134 and 125 kcal/kg, respectively, using the extrapolation method ([Kong and Adeola, 2014](#)). These results indicate that the energetic value of soybean hulls is relatively low, which is in accordance with views expressed by others ([Stein et al., 2008](#); [Wei et al., 2019](#)). Therefore, the dietary addition of soybean hulls generated calibration diets with a wide range of energetic values to eliminate leverage in developing ME and IVDE prediction equations. The actual IVDE values of calibration samples can be calculated by the concentrations of dietary ingredients and their IVDE values. A high degree of sensitivity is evident when the determined IVDE is similar to actual IVDE, which is in accordance with definitions of sensitivity described by others ([Ahmadi and Golian, 2010](#); [St-Pierre, 2015](#)). In our data, the range of determined IVDE was comparable with that of actual IVDE across 8 calibration samples, and the ratio of determined IVDE to actual IVDE was close to 1. Furthermore, the regression of determined IVDE on actual IVDE was consistent with the line of  $Y = X$ . These results indicate the IVDE determined by CCSDS is highly sensitive. The source of cereal grains differed across calibration diets, but other ingredients remained the same. The range of IVDE was very close to that of AME or AMEn across diets. A high correlation was also observed between IVDE and ME of diets. These results further support the conclusion that the sensitivity of IVDE determined with CCSDS is comparable to that established using in vivo techniques ([Bourdillon et al., 1990](#)).

A high correlation must exist between in vitro and in vivo values when establishing in vitro methods to predict digestibility of feedstuffs to gain acceptability among nutritionists ([Boisen and Eggum, 1991](#)). Furthermore, there must be minimal differences and similar ranges of in vitro and in vivo values across feed samples ([Valdes and Leeson, 1992](#); [Zhao et al., 2014](#); [Zhang et al., 2019](#)). The IVDE value determined in the present study was equal to 1.03 or 1.05 times of AME or AMEn value, and the range of IVDE (609 kcal/kg) was very close to that of AME (533 kcal/kg) or AMEn (525 kcal/kg) in the calibration diets. Moreover, IVDE was highly correlated with ME. The  $R^2$  for predicting AME and AMEn from IVDE was quite high in the current trial ( $R^2 = 0.9812$  and  $0.9821$  and RSD = 28 and 27 kcal/

kg). Across 71 diets, others have reported the ratio of IVDE to AMEn to be 1.04, and the ranges of IVDE or AMEn were 1,135 or 1,219 kcal/kg, respectively; the model to predict AMEn from IVDE had a  $R^2$  of 0.71 and RSD of 152 kcal/kg using manual, in vitro digestion processes (Valdes and Leeson, 1992). These findings suggest that the novel CCSDS method is superior to manual in vitro digestion method (Valdes and Leeson, 1992). Our results indicate that in vitro digestion progressed by CCSDS can simulate major in vivo digestion processes and accurately measure the variation in energetic values of diets and support our earlier findings (Zhao et al., 2014).

To further validate the accuracy of prediction models, others have used unknown samples which were absent in the calibration samples as validation samples (Meloche et al., 2014; Urriola et al., 2014). In general, the accuracy was validated by comparing the difference between predicted and determined values (Urriola et al., 2014; Zhao et al., 2014; Shi et al., 2015) or testing whether regression of determined on predicted values was consistent with the line of  $Y = X$  (Boisen and Fernández, 1997; Alvarenga et al., 2015). Others have established that the efficacy of prediction is satisfactory when predicted and determined ME differed by less than 100 kcal/kg (Valdes and Leeson, 1992) or regression models of determined on predicted ME overlapped with the line of  $Y = X$  (Boisen and Fernández, 1997). We observed comparable ratios of IVDE to ME for 8 calibration diets, similar ranges in IVDE and ME across diets, a difference of less than 100 kcal/kg between predicted and determined ME, and the regression of determined on predicted ME overlapped with the line of  $Y = X$ . These results further support the accuracy of the model to predict dietary ME from IVDE.

In conclusion, the IVDE of compound diets determined with CCSDS is highly sensitive. The IVDE measured with CCSDS is predictive of ME in diets. Therefore, IVDE determined with CCSDS is an effective method to predict the ME of diets for roosters, and it may offer benefits of being more efficient and cost-effective than in vivo techniques.

## ACKNOWLEDGMENTS

This project was financially supported by the Innovation Team of the Chinese Academy of Agricultural Sciences (ASTIP-IAS07) (Beijing, China) and fund of Wen's Food Group Co. Ltd. (2019-YF-06) (Guangdong, China).

## DISCLOSURES

The authors declare no conflicts of interest.

## REFERENCES

- Ahmadi, H., and A. Golian. 2010. Growth analysis of chickens fed diets varying in the percentage of metabolizable energy provided by protein, fat, and carbohydrate through artificial neural network. *Poult. Sci.* 89:173–179.
- Alvarenga, R. R., P. B. Rodrigues, M. G. Zangeronimo, E. C. Oliveira, F. C. M. Q. Mariano, E. M. C. Lima, A.A.P. Garcia, Jr, L. P. Naves, and N. B. S. Nardelli. 2015. Validation of prediction equations of energy values of a single ingredient or their combinations in male broilers. *Asian Australas. J. Anim. Sci.* 28:1335–1344.
- Association of Official Analytical Chemists (AOAC). 1990. *Official Methods of Analysis*. 15th ed. Assoc. Off. Anal. Chem., Washington, DC.
- Boisen, S., and B. O. Eggum. 1991. Critical evaluation of in-vitro methods for estimating digestibility in simple-stomach animals. *Nutr. Res. Rev.* 4:141–162.
- Boisen, S., and J. A. Fernández. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. *Anim. Feed Sci. Technol.* 68:277–286.
- Bourdillon, A., B. Carré, L. Conan, J. Duperray, G. Huyghebaert, B. Leclercq, M. Lessire, J. McNab, and J. Wiseman. 1990. European reference method for the in vivo determination of metabolizable energy with adult cockerels: reproducibility, effect of food intake and comparison with individual laboratory methods. *Br. Poult. Sci.* 31:557–565.
- Bryan, D. D. S. L., D. A. Abbott, and H. L. Classen. 2018. Development of an in vitro protein digestibility assay mimicking the chicken digestive tract. *Anim. Nutr.* 4:401–409.
- China Agricultural Industry Standard. 2004. Feeding standard of chicken (NY/T 33-2004). Ministry of Agriculture, Beijing, P. R. China.
- China National Standard. 2002. Method for Determination of Moisture, Crude Protein, Crude Fat, Crude Fiber, Lysine and Methionine in Feed-Near Infrared Reflectance Spectroscopy (GB/T 18868-2002). General Administration of Quality Supervision, Inspection and Quarantine, Beijing, P. R. China.
- Clunies, M., and S. Leeson. 1984. In vitro estimation of dry matter and crude protein digestibility. *Poult. Sci.* 63:89–96.
- Clunies, M., S. Leeson, and J. D. Summers. 1984. In vitro estimation of apparent metabolizable energy. *Poult. Sci.* 63:1033–1039.
- Farrell, D. J. 1978. Rapid determination of metabolizable energy of foods using cockerels. *Br. Poult. Sci.* 19:303–308.
- Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. *J. Nutr.* 65:587–603.
- Hill, F. W., D. L. Anderson, R. Renner, and L. B. Carew. 1960. Studies of the metabolizable energy of grain and grain products for chickens. *Poult. Sci.* 39:573–579.
- Institute of Animal Sciences of CAAS, China Feed-database Information Network Centre, and State Key Laboratory of Animal Nutrition 2019. Tables of feed composition and nutritive values in China. *China Feed* 21:97–107.
- Jha, R., and U. P. Tiwari. 2016. Rapid techniques for feed evaluation: scope and limitations. *Proc. NZ Poult. Industry Conf.* 13:84–102.
- Kaps, M., and W. Lamberson. 2004. *Biostatistics for Animal Science*. CABI-Publishing, Cambridge, UK.
- Kong, C., and O. Adeola. 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. *Asian Australas. J. Anim. Sci.* 27:917–925.
- Losada, B., P. García Rebollar, C. Álvarez, P. Cachaldora, M. A. Ibáñez, J. Méndez, and J. C. De Blas. 2010. The prediction of apparent metabolizable energy content of oil seeds and oil seed by-products for poultry from its chemical components, in vitro analysis or near-infrared reflectance spectroscopy. *Anim. Feed Sci. Technol.* 160:62–72.
- Losada, B., P. García Rebollar, P. Cachaldora, C. Álvarez, J. Méndez, and J. C. De Blas. 2009. A comparison of the prediction of apparent metabolizable energy content of starchy grains and cereal by-products for poultry from its chemical components, in vitro analysis or near-infrared reflectance spectroscopy. *Span. J. Agri.* 7:813–823.
- Mateos, G. G., L. Cámara, G. Fondevila, and R. P. Lázaro. 2018. Critical review of the procedures used for estimation of the energy content of diets and ingredients in poultry. *J. Appl. Poult. Res.* 28:506–525.
- Meloche, K. J., B. J. Kerr, N. Billor, G. C. Shurson, and W.A. Dozier, III. 2014. Validation of prediction equations for



- apparent metabolizable energy of corn distillers dried grains with solubles in broiler chicks. *Poult. Sci.* 93:1428–1439.
- Noblet, J., and Y. Jaguelin-Peyraud. 2007. Prediction of digestibility of organic matter and energy in the growing pig from an in vitro method. *Anim. Feed Sci. Technol.* 134:211–222.
- Pan, L., H. Ma, X. S. Piao, L. Liu, and D. F. Li. 2018. 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.* 235:43–49.
- Regmi, P. R., W. C. Sauer, and R. T. Zijlstra. 2008. Prediction of in vivo apparent total tract energy digestibility of barley in grower pigs using an in vitro digestibility technique. *J. Anim. Sci.* 86:2619–2626.
- Sakamoto, K., T. Asano, A. Furuya, and S. Takahashi. 1980. Estimation of in vivo digestibility with the laying hen by an in vitro method using the intestinal fluid of the pig. *Br. J. Nutr.* 43:389–391.
- Shi, C. X., Z. Y. Liu, M. Shi, P. Li, Z. K. Zeng, L. Liu, C. F. Huang, Z. P. Zhu, and D. F. Li. 2015. Prediction of digestible and metabolizable energy content of rice bran fed to growing pigs. *Asian Australas. J. Anim. Sci.* 28:654–661.
- Sibbald, I. R. 1976. A bioassay for true metabolizable energy in feedingstuffs. *Poult. Sci.* 55:303–308.
- Sol, C., L. Castillejos, S. López-Vergé, and J. Gasa. 2017. Prediction of the digestibility and energy contents of nonconventional by-products for pigs from their chemical composition and in vitro digestibility. *Anim. Feed Sci. Technol.* 234:237–243.
- Stein, H. H., L. L. Berger, J. K. Drackley, G. C. Fahey, Jr, D. C. Hernot, and C. M. Parsons. 2008. 18 - nutritional properties and feeding values of soybeans and their coproducts. Pages 613–660 in *Soybeans*. L. A. Johnson, P. J. White, and R. Galloway, eds. AOCS Press, Urbana, IL.
- St-Pierre, N. 2015. Statistical issues in nutritional modelling. Pages 62–72 in *Nutritional Modelling for Pigs and Poultry*. N. K. Sakomura, R. M. Gous, I. Kyriazakis, and L. Hauschild, eds. CABI, Boston, MA.
- Sturkie, P. D. 1976. *Avian Physiology*. 3rd ed. Springer-Verlag, New York, NY.
- Świąch, E. 2017. Alternative prediction methods of protein and energy evaluation of pig feeds. *J. Anim. Sci. Biotechnol.* 8:39.
- Urriola, P. E., M. Lia, B. J. Kerrb, and G. C. Shurson. 2014. Evaluation of prediction equations to estimate gross, digestible, and metabolizable energy content of maize dried distillers grains with solubles (DDGS) for swine based on chemical composition. *Anim. Feed Sci. Technol.* 198:196–202.
- Valdes, E. V., and S. Leeson. 1992. Measurement of metabolizable energy in poultry feeds by an in vitro system. *Poult. Sci.* 71:1493–1503.
- Wei, J., M. Xie, Q. Zhang, J. Tang, and S. Hou. 2019. Correlation between enzyme hydrolysate gross energy and metabolizable energy of soybean meal for ducks. *Chin. J. Anim. Nutr.* 31:1623–1629.
- Wiseman, J., N. T. Nicol, and G. Norton. 2000. Relationship between apparent metabolizable (AME) values and in vivo/in vitro starch digestibility of wheat for broilers. *World Poult. Sci. J.* 56:305–318.
- Yegani, M., and D. R. Korver. 2012. Review: prediction of variation in energetic value of wheat for poultry. *Can. J. Anim. Sci.* 92:261–273.
- Yegani, M., M. L. Swift, R. T. Zijlstra, and D. R. Korver. 2013. Prediction of energetic value of wheat and triticale in broiler chicks: a chick bioassay and an in vitro digestibility technique. *Anim. Feed Sci. Technol.* 183:40–50.
- Zhang, L., F. Zhao, H. Zhang, G. Z. Bian, Y. M. Wang, X. Yang, and H. Li. 2019. Validation of in vitro digestion using simulated small intestinal fluid with specific digestive activity to predict the metabolizable energy of feed ingredients for duck. *Poult. Sci.* 98:1280–1287.
- Zhao, F., Y. Liu, and H. F. Zhang. 2010. Effects of dietary protein source on the composition of small intestinal fluid of layer-type cockerels. *Chin. J. Anim. Nutr.* 22:1279–1285.
- Zhao, F., L. Q. Ren, B. M. Mi, H. Z. Tan, J. T. Zhao, H. Li, H. F. Zhang, and Z. Y. Zhang. 2014. Developing a computer-controlled simulated digestion system to predict the concentration of metabolizable energy of feedstuffs for rooster. *J. Anim. Sci.* 92:1537–1547.