Original Research Article

Accuracy of predicting metabolizable energy from in vitro digestible energy determined with a computer-controlled simulated digestion system in feed ingredients for ducks

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ABSTRACT

Two experiments were conducted to study the accuracy of predicting true metabolizable energy (TME) of ingredients for ducks from in vitro digestible energy (IVDE) determined with a computer-controlled simulated digestion system. Experiment 1 was to establish TME prediction models from the IVDE of 9 energy feed ingredients and 12 protein feed ingredients using regression analysis. Experiment 2 was to validate the accuracy of the predicted ME of 10 ingredients randomly selected from Exp. 1. Ten diets were formulated with 2 to 6 of 10 ingredients. Dietary in vivo TME values were compared with calculated values based on the TME predicted in Exp. 1. In Exp. 1, the correlation coefficients between TME and IVDE were 0.9339 (P < 0.05) in 9 energy feed ingredients and 0.8332 (P < 0.05) in 12 protein feed ingredients. No significant difference was observed on the slope and intercept of TME regression models between 9 energy feed ingredients and 12 protein feed ingredients. Therefore, the regression model of TME on IVDE for 21 feed ingredients was TME = 0.7169 × C2 IVDE + 1,224 (R² = 0.7542, P < 0.01). Determined and predicted TME differed by less than 100 kcal/kg of DM in 11 ingredients, and the difference ranged from 100 to 200 kcal/kg of DM in 5 ingredients. However, the difference between determined and predicted TME varied from 410 to 625 kcal/kg of DM in rice bran, rapeseed meal, corn gluten meal, and citric acid meal. In Exp. 2, the determined and calculated TME were comparable (3,631 vs. 3,639 kcal/kg of DM) and highly correlated (r = 0.9014; P < 0.05) in 10 diets. Determined and calculated TME differed by less than 100 kcal/kg of DM in 7 diets and by 106 to 133 kcal/kg of DM in 3 diets. These results have demonstrated that TME can be accurately predicted from IVDE in most feed ingredients, but it is less accurate for rice bran, rapeseed meal, corn gluten and citric acid meal.

1. Introduction

In vitro simulated digestion is an efficient, simple, and precise method to evaluate feed quality, but it is imperative that methods are validated and accurate for particular species. The in vitro digestion procedure developed by Boisen and Fernandez (1997) is commonly used to predict available energy of feed ingredients for pigs (Woyengo et al., 2016; Sol et al., 2017). But these simulated digestion processes for swine are often used to represent poultry (Yegani et al., 2013), which may not be the best approach. Recently, a computer-controlled simulated digestion system (CCSDS) was developed to determine in vitro digestible energy (IVDE) to predict ME of corn for ducks using simulated intestinal fluid that matches the in vivo composition in ducks (Zhao et al., 2014). The ME was highly correlated with the IVDE, as determined by this in vitro digestion system, in 9 feed ingredients (Zhang et al., 2019) and 6 diets (Wei et al., 2020) for ducks. Therefore, this novel in vitro digestion method can rapidly determine the energy digestibility of some ingredients for ducks, but it must be validated across a greater
variety of feed ingredients. In previous studies, the extent of the correlation of in vitro and in vivo digestion has varied (Cone and Van Der Poel, 1993; Boisen and Fernandez, 1997; Regmi et al., 2009). The correlation coefficients are dependent on the precision of in vivo and in vitro methods and the range of determined values in calibration samples. Several studies have developed linear models of ME with IVDE based on the values of all samples together (Valdes and Leeson, 1992; Zhao et al., 2014; Zhang et al., 2019), which is subjective because all feeds are assumed to have a consistent relationship between in vitro and in vivo values. In consideration of these problems and to validate the accuracy of predicting ME from in vitro digestion of a wide range of feed ingredients, the objective of this study was to 1) test whether regression models of ME from IVDE was similar between 9 energy feed ingredients and 12 protein feed ingredients to decide to pool or separate the samples to establish models of ME on IVDE and 2) validate the accuracy of predicted ME of feed ingredients by comparing dietary in vivo ME and calculated ME from the predicted ME of feed ingredients and its concentration in diets.

2. Materials and methods

Experimental procedures were approved by the animal care and welfare committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China).

2.1. Experimental design

Two experiments were conducted in this study. Experiment 1 was to establish prediction equations for true metabolizable energy (TME) from the IVDE of 9 energy feed ingredients and 12 protein feed ingredients. The slopes and intercepts were compared across ingredients in these two prediction models. If there was no significant difference in each of the slopes and intercepts, the 21 feed ingredients were pooled to establish the TME prediction models for IVDE. Each sample was determined for TME in 4 replicates of 3 ducks, and for IVDE in 5 replicates of 1 digestive tube. The relationship of TME and IVDE was evaluated by regression analysis. Experiment 2 was to validate the accuracy of predicted TME in Exp. 1. Ten out of the 21 feed ingredients were randomly selected to formulate 10 diets with 2 to 6 ingredients. Dietary calculated TME was the sum of predicted TME of individual ingredient from the model established in Exp. 1 multiplying its concentration in the diet. The in vivo TME value of each diet was determined in 4 replicates of 3 ducks. The in vivo and calculated TME values of diets were compared to test the accuracy of predicted TME in Exp. 1.

2.2. Feed ingredients and experimental diets

A total of 21 samples were obtained from a feed company in China (Newhope Liuhe Co. Ltd., Beijing, China). The samples represented a variety of ingredients (Table 1) common to ducks in China, including 9 energy feedstuffs (corn 1, corn 2, wheat 1, wheat 2, barley, sorghum, wheat flour, rice bran, and rice bran meal) and 12 protein feedstuffs (wheat middling, wheat bran, soybean meal, cottonseed meal, peanut meal 1, peanut meal 2, rapeseed meal, corn distillers dried grains with solubles (DDGS) 1, corn DDGS 2, corn gluten meal, citric acid meal (a by-product in citric acid production from the fermentation of corn or cassava), and monosodium glutamate meal (a by-product in the extraction of monosodium glutamate, composed of a high content of non-protein nitrogen). In Exp. 1, diets 1 to 7 were formulated with the test ingredient and premix of minerals and vitamins, whereas the diets 8 to 21 were formulated with corn 2, the test ingredient, and premix (Table 2). In Exp. 2, validation diets 1 to 3 were composed of 2 to 4 feed ingredients of corn 2, soya bean meal, wheat 1, and wheat bran (Table 3). Validation diets 4 to 8 were composed of 2 to 6 feed ingredients of corn 2, corn DDGS 2, rice bran, cottonseed meal, rapeseed meal, and wheat bran (Table 3). Validation diets 9 and 10 were composed of 3 or 4 feed ingredients of corn 2, wheat 1, sorghum, and barley (Table 3). About 5 kg of each diet was ground and pelleted. All pelleted diets were air-dried until the water content was less than 14% for the bioassay.

2.3. In vivo true metabolizable energy assay

The ME values were determined for 21 diets in Exp. 1 and 10 diets in Exp. 2 at the experimental base of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China). The procedure for the TME assay followed the China Agricultural Industry Standard (NY/T 2122-2012) and Zhao et al. (2008). Briefly, after 5 d adaptation period, the drakes were deprived of feed for 36 h and force-fed with 60 g experimental diet. After force-feeding, excreta was collected for 36 h to determine the apparent metabolizable energy (AME). The endogenous energy loss (EEL) was determined by excluded energy in the feed-deprived drakes. The TME was calculated by AME plus the EEL.

A total of 24 determinations for 21 diets and 3 EEL were randomly assigned to 6 metabolic trials in Exp. 1. Another 12 determinations for 10 diets and 2 EEL were randomly assigned to 3 metabolic trials in Exp. 2. In each metabolic trial, 48 Cherry Valley drakes (average BW = 3.7 kg) were divided into 4 groups. Each group contained 4 replicates of 3 drakes for a single determination.

2.4. In vitro digestible energy determined by the computer-controlled simulated digestion system

The IVDE of feed ingredients and diets was determined according to the procedure described by Zhao et al. (2014). The digestive chamber, simulated gastric fluid, simulated small intestinal fluid, gastric buffer solution, and small intestinal buffer solution were all prepared in accordance with the procedures reported by Zhao et al. (2014). The CCSDS is illustrated in Fig. 1. Before the run, 2 g of grain feed ingredient or diet, or 1 g of another feed ingredient and 20 mL of simulated gastric fluid, were added into each digestion chamber. Two groups of 5 replicated digestive chambers were fixed on a platform and connected with middle silicone tubes, and then fixed in the reactor cabinet (1 in Fig. 1). The solution entry of the first digestion chamber (2 in Fig. 1) was connected to buffer bottles (3 in Fig. 1) by electric valves (4 in Fig. 1) and a peristaltic pump (5 in Fig. 1) to circulate the buffer solution. The solution exit of the fifth digestion chamber was connected to the buffer bottle (3 in Fig. 1) by electric valves. Entry of the simulated small intestinal fluid in each digestion chamber was connected to a small bottle in the low temperature storage cabinet (6 in Fig. 1) for digestive fluid by small silicone tubes and a multiple channel peristaltic pump (7 in Fig. 1). Bottles (3 in Fig. 1) filled with buffer solution were fixed in a water-warmed bath (8 in Fig. 1). After the gastric or small intestine digestion, 1,500 mL of deionized water was pumped into each bottle (9 in Fig. 1), and then pumped into the digestive chambers and circulated for cleaning the digestion by-product. A shaker (10 in Fig. 1) responsible for mixing the sample was fixed under the platform for the digestive chambers in the reactor cabinet (1 in Fig. 1). The reactor cabinet was maintained at 42 °C using a warm-air recycling system (11 in Fig. 1) composed of a heat pipe and a computer-controlled circuit box (12 in Fig. 1). The parameters included 4 h for gastric digestion, 7.5 and 7.5 h for upper- and lower-intestinal digestion respectively, 2 mL of concentrated simulated small intestinal fluid, 42 °C for the buffer solution and reactor cabinet, 180 rpm of shaking speed, an
emptying solution procedure and 3 replicated wash procedures after gastric digestion, and 6 replicated wash procedures after small intestine digestion was set in the computer to automatically process in vitro digestion for ducks. After completing the simulated digestion, the undigested residues were dried, defatted, and then dried to constant weight.

2.5. Chemical analysis

Samples were ground finely in a laboratory mill that was fitted with a 0.3-mm mesh screen prior to chemical analysis. The DM content (Method 930.15; AOAC, 2007) was determined by an oven at 105 °C for 5 h. Feed ingredients were analyzed for CP (Method 990.03; AOAC, 2007), ether extract (method 920.39; AOAC, 2007), crude fiber (method 978.10; AOAC, 2007), and ash (method 942.05; AOAC, 2007). Diet, excreta, and residue samples were analyzed for gross energy (GE) using an IKA C2000 adiabatic calorimeter (GmbH & Co. KG, Staufen, Germany) with benzoic acid as the calibration standard.

2.6. Data calculation and statistical analysis

The TME of corn, wheat, barley, sorghum, and wheat flour was calculated as follows: TME (kcal/kg) = (E_in - E_out + EEL/E_in × GE), where E_in is the GE intake (kcal) in the diet, E_out is the GE output (kcal) in the feces, EEL is the EEL (kcal) in the feces, and GE is the GE per unit weight (kcal/kg) in the test feed ingredient.

The TME of other feed ingredients was calculated by a difference method according to the following formulas described by Woyengo et al. (2010): TRGE (%) = TRGE_cor (TRGE - TRGE/EC) / (EC × GE), TME (kcal/kg) = TRGE × GE/r, where r and t represent the test feed and experimental diet, respectively. Additionally, TRGE, TRGE_cor, and TRGE are the true retention rate (%) of GE for the test ingredient, corn 2, and experimental diet, respectively, and EC denotes the proportion (%) of GE from the test feed ingredient in the experimental diet.

The IVDE was calculated as follows: IVDE (kcal/kg) = [(E_t - E_r) + E_r] / W_t, where t, r and e represent the test feed, defatted residue of feed and dry residue of enzyme, respectively. Additionally, E_t is the GE (kcal) of the test feed ingredient added in each digestion chamber, E_r is the GE (kcal) of the defatted residue output in each digestion chamber, E_e is the GE (kcal) of the dry residue of digestive enzymes, and W_t is the DM weight (kg) of the test feed ingredient added in each digestion chamber.

Means and ranges for IVDE or ME were calculated with the MEANS procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). The simple correlation between TME and IVDE was analyzed using the CORR procedure. Models of TME predicted from IVDE were developed by the REG procedure. Significant differences in intercepts or slopes of the TME prediction models between 9 energy feed ingredients and 12 protein feed ingredients were tested using the GLM procedure according to the statistical principle described by Kaps and Lamberson (2017).

3. Results

3.1. Correlation between the in vitro digestible energy and true metabolizable energy of feed ingredients

As shown in Table 4, the mean IVDE was 3,561 kcal/kg of DM in 9 energy feed ingredients, and ranged from 2,007 to 4,085 kcal/kg of DM. The mean TME was 3,731 kcal/kg of DM, ranging from 2,568 to 4,104 kcal/kg of DM. TME and IVDE were highly correlated (r = 0.9339, P < 0.05). The mean IVDE of the 12 protein feed ingredients was 3,255 kcal/kg of DM (range: 2,324 to 4,682 kcal/kg of DM). The mean TME of the protein ingredients was 3,592 kcal/kg of DM (range: 2,760 to 4,991 kcal/kg of DM). A correlation coefficient of 0.8332 (P < 0.05) was observed between TME and IVDE. The correlation coefficient (r = 0.8684, P < 0.05) between TME and IVDE for all 21 feed ingredients pooled was lower than that in the 9 energy feed ingredients (r = 0.9339), but greater than that in the 12 protein feed ingredients (r = 0.8332).

3.2. Regression models of true metabolizable energy against in vitro digestible energy for feed ingredients

The models to predict TME from IVDE in 9 energy feed ingredients and 12 protein feed ingredients are shown in Fig. 2:
Table 2
The composition of the experimental diets (Exp. 1, DM basis, %).

| Feed ingredient                  | Experimental diet |
|----------------------------------|-------------------|
|                                  | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    | 16    | 17    | 18    | 19    | 20    | 21    |
| Corn 1                           | 95.15 |
| Corn 2                           | 95.19 |
| Wheat 1                          | 95.22 |
| Wheat 2                          | 95.20 |
| Barley                           | 95.25 |
| Sorghum                          | 95.15 |
| Wheat flour                      | 95.10 |
| Rice bran                        | 95.10 |
| Rice bran meal                   | 40.16 |
| Wheat middling                   | 39.88 |
| Wheat bran                       | 39.52 |
| Soybean meal                     | 39.01 |
| Cottonseed meal                  | 39.65 |
| Peanut meal 1                    | 40.39 |
| Peanut meal 2                    | 40.53 |
| Rapesed meal                     | 40.67 |
| Corn DDGS 1                      | 40.67 |
| Corn DDGS 2                      | 41.09 |
| Corn gluten meal                 | 39.99 |
| Citric acid meal                 | 39.88 |
| Monosodium glutamate meal        | 40.53 |
| Premix¹                          | 41.24 |
|                                  | 4.85  | 4.81  | 4.78  | 4.80  | 4.75  | 4.85  | 4.80  | 4.78  | 4.80  | 4.83  | 4.87  | 4.82  | 4.76  | 4.75  | 4.74  | 4.71  | 4.79  | 4.81  | 4.76  | 4.76  | 4.70  |

DDGS – distillers dried grains with solubles.

¹ The premix provided the following per kilogram of diets (as-fed basis): monocalcium phosphate, 18.0 g; limestone, 19.0 g; sodium chloride, 4.0 g; vitamin A 2,500 IU; vitamin D₃ 400 IU; vitamin E 10 IU; vitamin K₃ 0.5 mg; vitamin B₁ 1.8 mg; vitamin B₂ 1 mg; vitamin B₆ 3 mg; vitamin B₁₂ 7 μg; pantothenic acid 11 mg; nicotinic acid 55 mg; folic acid 0.5 mg; biotin 0.12 mg; choline chloride 750 mg; Cu (as copper sulfate) 8 mg; Fe (as ferrous sulfate) 80 mg; Zn (as zinc sulfate) 40 mg; Mn (as manganese sulfate) 60 mg; Se (as sodium selenite) 0.15 mg; I (as potassium iodide) 0.35 mg.
TME = 0.7451 × IVDE + 1,078 \left( R^2 = 0.8722, \text{ residual SD [RSD]} = 187 \text{ kcal/kg, } P < 0.01 \right) \text{ and TME} = 0.7255 \times \text{IVDE} + 1,230 \left( R^2 = 0.6943, \text{ RSD} = 332 \text{ kcal/kg, } P < 0.01 \right). \text{ No significant difference was observed on the slopes} \left( P = 0.9256 \right) \text{ or intercepts} \left( P = 0.5022 \right) \text{ between the TME prediction models of 9 energy feed ingredients and 12 protein feed ingredients. Therefore, data from all 21 feed ingredients were pooled to establish the model as TME} = 0.7169 \times \text{IVDE} + 1,224 \left( R^2 = 0.7542, \text{ RSD} = 270 \text{ kcal/kg, } P < 0.01, \text{ Fig. 3} \right).
The accuracy of predicting the TME from the IVDE in feed ingredients for ducks (Exp. 1).

Table 5: The accuracy of predicting the TME from the IVDE in feed ingredients for ducks (Exp. 1).

| Feed ingredient    | IVDE, kcal/kg | TME, kcal/kg | Predicted<sup>1</sup> | Difference<sup>2</sup> | CI<sup>3</sup> |
|--------------------|---------------|--------------|------------------------|------------------------|------------|
|                    | Determined    |              |                        |                        |            |
| Corn 1             | 3,762         | 4,055        | 3,921                  | 134                    | 3,777 to 4,064 |
| Corn 2             | 3,811         | 4,029        | 3,956                  | 73                     | 3,807 to 4,105 |
| Wheat 1            | 3,731         | 3,799        | 3,899                  | –100                   | 3,758 to 4,039 |
| Wheat 2            | 3,665         | 3,919        | 3,851                  | 68                     | 3,716 to 3,986 |
| Barley             | 3,340         | 3,557        | 3,618                  | –61                    | 3,495 to 3,742 |
| Sorghum            | 3,850         | 4,057        | 3,984                  | 73                     | 3,831 to 4,137 |
| Wheat flour        | 4,085         | 4,104        | 4,153                  | –49                    | 3,968 to 4,337 |
| Rice bran          | 3,794         | 3,487        | 3,944                  | –457                   | 3,779 to 4,091 |
| Rice bran meal     | 2,007         | 2,568        | 2,663                  | –95                    | 2,365 to 2,960 |
| Wheat middling     | 3,302         | 3,330        | 3,591                  | –61                    | 3,467 to 3,715 |
| Wheat bran         | 2,324         | 2,760        | 2,890                  | –130                   | 2,648 to 3,132 |
| Soybean meal       | 3,528         | 3,836        | 3,753                  | 83                     | 3,627 to 3,829 |
| Cottonseed meal    | 2,659         | 3,125        | 3,130                  | –5                     | 2,941 to 3,319 |
| Peanut meal 1      | 3,591         | 3,626        | 3,798                  | –172                   | 3,669 to 3,928 |
| Peanut meal 2      | 3,978         | 3,928        | 4,076                  | –48                    | 3,906 to 4,245 |
| Rapeseed meal      | 3,277         | 2,958        | 3,573                  | –615                   | 3,448 to 3,698 |
| Corn DDGS 1        | 3,234         | 3,691        | 3,542                  | 149                    | 3,416 to 3,669 |
| Corn DDGS 2        | 3,156         | 3,760        | 3,487                  | 273                    | 3,355 to 3,617 |
| Corn gluten meal   | 4,082         | 4,991        | 4,581                  | 410                    | 4,297 to 4,863 |
| Citric acid meal   | 2,487         | 3,632        | 3,007                  | 625                    | 2,791 to 3,222 |
| Monosodium glutamate meal | 2,840 | 3,261 | 3,260 | 1 | 3,096 to 3,423 |
| Mean               | 3,386         | 3,651        | 3,651                  | –                  | 3,386 to 3,651 |
| SEM                | 61            | 59           |                        |                        |            |
| ARD<sup>4</sup>, % | 3.345         | 3,624        | 3,622                  | 7.02                   |            |
| Mean<sup>5</sup>   | 3,345         | 3,624        | 3,622                  | 7.02                   |            |
| ARD, %             | 3.24          |              |                        |                        |            |

IVDE – in vitro digestible energy; TME – true metabolizable energy; DDGS – distillers dried grains with solubles.
1. The values were predicted according to TME = 0.7169 × IVDE + 1.224 (R² = 0.7542, RSD = 270 kcal/kg, P < 0.01).
2. Difference = Determined - Predicted.
3. Confidence intervals (CI), 95% confidence intervals for the mean value of the predicted TME.
4. Average relative differences (ARD) = \( \frac{100 \times \sqrt{\frac{\sum (\text{Determined} - \text{Predicted})^2}{\text{Mean of the determined values}}}}{\text{Mean of the determined values}} \)
5. The values that rice bran, rapeseed meal, corn gluten meal and citric acid meal are excluded from the above 21 feed ingredients.
The estimates of the TME of corn, sorghum, and wheat was similar (with differences of <123 kcal/kg of DM). The TME of soybean meal was greater (with differences of >701 kcal/kg of DM). The considerable difference in the TME of soybean meal between the in vivo and in vitro values (Valdes and Leeson, 1992; Regmi et al., 2009) indicates that the relationship between TME and IVDE may be different. We also observed that the TME of peanut meal 1, corn DDGS, citric acid meal, and wheat middling were similar to each other (with differences of <230 kcal/kg of DM) but all were less than that of soybean meal. However, the IVDE of these 4 ingredients varied considerably (range: 1,140 kcal/kg of DM). Similarly, the cottonseed meal, rapeseed meal, and monosodium glutamate meal were similar in terms of TME (with differences of <303 kcal/kg of DM), but there was a wider range amongst the IVDE of these 4 ingredients (range: 618 kcal/kg of DM). These results indicated the relationship between TME and IVDE was inconsistent in some protein ingredients. The IVDE was highly lower than the TME of citric acid meal, but the IVDE was greater than the TME of rapeseed meal with a high fat content. As a result, the correlation between TME and IVDE deviated for these 2 ingredients relative to the others. Consequently, the correlation coefficient between TME and IVDE was lower in the 12 protein feed ingredients than in the 9 energy feed ingredients.

| Diet | IVDE, kcal/kg | TME, kcal/kg | Determined | Calculated | Difference | CI |
|------|--------------|--------------|------------|------------|------------|---|
| 1    | 3,482        | 3,834        | 3,728      | 106        | 3,683 to 3,832 |
| 2    | 3,492        | 3,807        | 3,720      | 87         | 3,674 to 3,818 |
| 3    | 3,178        | 3,543        | 3,522      | 21         | 3,376 to 3,549 |
| 4    | 3,438        | 3,733        | 3,649      | 84         | 3,588 to 3,700 |
| 5    | 3,480        | 3,657        | 3,660      | –3         | 3,603 to 3,717 |
| 6    | 3,315        | 3,468        | 3,601      | –133       | 3,516 to 3,635 |
| 7    | 3,262        | 3,497        | 3,577      | –80        | 3,475 to 3,607 |
| 8    | 3,104        | 3,324        | 3,440      | –116       | 3,220 to 3,470 |
| 9    | 3,523        | 3,758        | 3,770      | –12        | 3,726 to 3,910 |
| 10   | 3,433        | 3,684        | 3,727      | –43        | 3,682 to 3,830 |
| Mean | 3,371        | 3,631        | 3,639      | –9         |                 |
| SEM  | 22           | 27           |            |            |                 |
| ARD, % | 0.9053*     | 0.9014*      | 2.24       |            |                 |
| r    |              |              |            |            |                 |
| T-test, P value |              |              |            |            |                 |
| P-value |              |              |            |            |                 |
| Slope |              |              | 1.4323     |            |                 |
| R²   |              |              | 0.8124     |            |                 |
| P-value |              |              | 0.0004     |            |                 |

IVDE = in vitro digestible energy; TME = true metabolizable energy.

1 The values of 10 diets were calculated according to the predicted TME of the feed ingredient and its concentration in the diet.
2 Difference = Determined - Predicted
3 Confidence intervals (CI), 95% confidence intervals for the mean value of the predicted TME.
4 Average relative difference (ARD) = \( \frac{1}{n} \sum_{i=1}^{n} \left( \frac{\text{Determined} - \text{calculated}}{\text{calculated}} \right) \times 100\%
5 Significant differences between determined TME and calculated TME of 10 diets were tested using paired T-test procedure.
6 Regression of determined on calculated TME.
7 Significance of the difference between the intercept and 0 in the regression model.
8 Significance of the difference between the slope and 1 in the regression model.
9 Significance of regression model.

In the 12 protein feed ingredients, the TME and IVDE were highly correlated with TME in 9 energy feed ingredients (Karunaratne et al., 2018; Wei et al., 2019, 2020), but also that TME was influenced by different bioassay procedures. The current results indicated the TME and IVDE were highest in wheat flour, but lowest in rice bran amongst the 9 energy feed ingredients. The TME of sorghum and corn were relatively close to the IVDE, but the IVME was slightly higher than the IME for wheat 1, and obviously higher for barley. This variation was in accordance to that reported by the Chinese Feed Database (Institute of Animal Sciences of CAAS, 2019), as well as to the study of Ragland et al. (1997), Hong et al. (2002), and Zhang et al. (2019). Thus, IVDE was highly correlated with TME in 9 energy feed ingredients (r = 0.9339). In the 12 protein feed ingredients, the TME and IVDE were highest in corn gluten meal and lowest in wheat bran. The TME and IVDE of peanut meal 2 were higher than for soybean meal. The TME of peanut meal 1, corn DDGS, citric acid meal, and wheat middling were similar to each other (with differences of <230 kcal/kg of DM) but all were less than that of soybean meal. However, the IVDE of these 4 ingredients varied considerably (range: 1,140 kcal/kg of DM). Similarly, the cottonseed meal, rapeseed meal, and monosodium glutamate meal were similar in terms of TME (with differences of <303 kcal/kg of DM), but there was a wider range amongst the IVDE of these 4 ingredients (range: 618 kcal/kg of DM). These results indicated the relationship between TME and IVDE was inconsistent in some protein ingredients. The IVDE was highly lower than the TME of citric acid meal, but the IVDE was greater than the TME of rapeseed meal with a high fat content. As a result, the correlation between TME and IVDE deviated for these 2 ingredients relative to the others. Consequently, the correlation coefficient between TME and IVDE was lower in the 12 protein feed ingredients than in the 9 energy feed ingredients.

### 4.2. Establishing a true metabolizable energy prediction model from in vitro digestible energy

Cone and Van Der Poel (1993) developed unique regressions between ileal and in vitro CP digestibility for each of concentrated feeds, pea, rapeseed products, and soybean products. Wei et al. (2019) selected 5 of 11 corn DDGS of variable IVDE to establish a model to predict TME from IVDE. However, other researchers developed only one model for all samples to predict in vivo values from in vitro values (Valdes and Leeson, 1992; Regmi et al., 2009; Zhao et al., 2014). Consequently, the establishment of prediction model is subjective. It is challenging to assess whether regression models for predicting in vivo from in vitro values are statistically valid and whether they can represent multiple types of feed ingredients.
ingredients. In this study, the prediction models of TME from IVDE between 9 energy feed ingredients and 12 protein feed ingredients did not differ in slopes or intercepts. This indicates that the relationship between TME and IVDE is the same for both energy and protein ingredients. Consequently, the 21 feed ingredients could be combined to establish a regression model of TME on IVDE. In the CCSDS, pepsin, trypsin, and chymotrypsin were responsible for protein digestion; amylase was responsible for starch digestion. The digestibility of fat was estimated by ethanol extract (Zhao et al., 2014). The relationship between TME and IVDE was similar for both energy and protein feed ingredients, because of the specificity of the digestive enzymes for each of the chemical structures.

4.3. Accuracy of predicted true metabolizable energy for feed ingredients

The RSD of the regression model to predict ME from IVDE (270 kcal/kg) for all 21 feed ingredients was greater than that of the TME prediction models from IVDE for the corn samples and the 10 feed ingredients (Zhang et al., 2019). The wider range of IVDE and TME of the 21 feed ingredients used in the current study could increase the RSD. Accordingly, this RSD was numerically similar to the model that predicted AME from IVDE determined with manual in vitro digestion for 94 grains and by-products (265 kcal/kg of DM; Losada et al., 2009), but it was less than that for 52 oilseed and by-products (379 kcal/kg of DM; Losada et al., 2010). In the current study, the difference between the determined and predicted TME was less than 100 kcal/kg of DM in 52.4% of samples, ranging from 100 to 200 kcal/kg of DM in 23.8% of samples, and differing by more than 200 kcal/kg of DM in the remaining 23.8% of samples. This accuracy was better than that of Valdes and Leeson (1992), where the determined and predicted AME differed by less than 100 kcal/kg of DM in 42.2% of samples, ranging from 100 to 200 kcal/kg of DM in 28.2% of samples, and differing by more than 200 kcal/kg of DM in the remaining 29.6% of samples.

The substitution method of TME bioassay determines the TME of both basal and experimental diets, and this leads to more errors when determining the TME of test ingredients. The range (maximum - minimum) of TME in 4 replicates for each sample was 139 kcal/kg of DM, 192 kcal/kg of DM, 122 kcal/kg of DM, and 111 kcal/kg of DM in metabolic trials to directly determine the TME of corn for ducks in the research of Adeola et al. (1997), King et al. (1997), Ragland et al. (1997), and Adeola (2003), respectively. Adedokun and Adeola (2005) also described a high standard error (190 to 457 kcal/kg of DM) of AME for meat and bone meal in ducks when using the substitution method. Therefore, the ME values of feed ingredients are more precise when they are determined by the direct method rather than by the substitution method. In this study, the TME of corn, wheat, barley, sorghum, and wheat flour was determined using the direct method, but the substitution method was used for other samples due to poor palatability issues or the test ingredients being unable to be fed for a long enough period of time to determine the digestibility. As a result, there was less error in the TME and more homogeneity between the determined and predicted TME for energy ingredients compared to protein ingredients. In the 12 protein ingredients, a substantial difference between the determined and predicted TME was observed in rice bran, rapeseed meal, corn gluten meal, and citric acid. This indicated that in vitro digestion for these 4 ingredients may not be consistent with the other 17 ingredients.

The idea that the sum of digestible nutrients in a complete diet is equal to the sum of those from the single ingredients is known as the additivity rule (Hong et al., 2002). This concept is usually used to validate a new method of determining ME and amino acid digestibility in feed ingredients (Sibbald, 1977; Cowieson et al., 2019; Osho et al., 2019). Accordingly, we used the additivity rule to validate the accuracy of the predicted TME for feed ingredients. Differences of less than 100 kcal/kg of DM between the determined and calculated dietary ME were considered to be of an acceptable accuracy according to the error in the bioassay of ME (Valdes and Leeson, 1992). Many unconventional feed ingredients are used to formulate diets for ducks in China, but grains and soybean meal account for the majority of the diets. In this study, validation diets 1 to 3 and 9 to 10 contained corn, soybean meal, wheat bran, wheat, barley, and sorghum. The determined and calculated TME differed by less than 106 kcal/kg of DM, which indicated that the TME predicted from the regression model was accurate for these 6 ingredients. Diets 4 and 5 contained corn, corn DDGS 2, and cottonseed meal. The data in Exp. 1 showed that the predicted TME was similar to the determined TME in corn and cottonseed meal, although the predicted TME was lower than the determined TME in corn DDGS 2. The determined and calculated TME of validation diets 4 and 5 differed by less than 100 kcal/kg of DM, which indicated that the predicted TME for corn DDGS 2 did not affect the accuracy of the calculated dietary TME. Validation diets 6 to 8 contained corn, cottonseed meal, rice bran, rapeseed meal, corn DDGS 2, and wheat bran. Data in Exp. 1 showed that the predicted TME exceeded the determined TME in rice bran and rapeseed meal. Accordingly, the calculated TME was greater than the determined TME in validation diets 6 to 8. These results indicated that the predicted TME values were overestimated for rice bran and rapeseed meal. The high correlation coefficient (r > 0.90) between the determined TME and IVDE or calculated TME values in 10 validation diets indicated that the changes in the IVDE and calculated TME values were consistent with the variation of in vivo TME. Our study showed that the difference between the determined and calculated TME was less than 133 kcal/kg of DM in all 10 validation diets, whereas the results of Valdes and Leeson (1992) showed differences of less than 200 kcal/kg of DM in 70.4% of samples. This further indicated that the CCSDS was more accurate than manual in vitro digestion when predicting the ME for ducks.

5. Conclusion

Our results showed that the determined and predicted TME was highly correlated for 9 energy ingredients and 12 protein ingredients, respectively. Additionally, no difference was observed on the slopes or intercepts between the prediction model of energy and protein ingredients, which implied that the prediction model can be established for all 21 feed ingredients together. The prediction model was further validated through 10 diets formulated with the above feed ingredients. Further studies should focus on optimizing the process of CCSDS for ingredients with a high fat or protein content, thus, due to the less accurately predicted TME of rice bran, rapeseed meal, corn gluten and citric acid meal.

Author contributions

Yuming Wang: Conceptualization, Methodology, Project administration, Formal analysis, Writing - Original draft. Liting Yin: Investigation, Formal analysis. Hu Zhang: Investigation, Project administration. Ke Li: Funding acquisition, Methodology, Project administration. Dailin Li: Validation, Resources. Feng Zhao: Conceptualization, Supervision, Project administration, Writing - Review & Editing.
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 SA, Adeola O. Apparent metabolizable energy value of meat and bone meal for white Pekin ducks. Poultry Sci 2005;84:1539–946.
Adeola O. Energy value of feed ingredients for white Pekin ducks. Int J Poultry Sci 2003;2:318–23.
Adeola O, Ragland D, King D. Feeding and excreta collection techniques in metabolizable energy assays for ducks. Poultry Sci 1997;76:728–32.
Association of Official Analytical Chemists (AOAC). Official methods of analysis. 18th ed. MD: AOAC Int. Gaithersberg; 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.
Cone JW, Van Der Poel AFB. Prediction of apparent ileal protein digestibility in pigs with a two-step in vitro method. J Sci Food Agric 1993;62:393–400.
Cowieson A, Sorbana JO, Pappenberg G, Abdolla M, M, T.R, Moors FF, Ravindran V. Additivity of apparent and standardized ileal amino acid digestibility of corn and soybean meal in broiler diets. Poultry Sci 2019;98:3722–8.
Hong D, Ragland D, Adeola O. Additivity and associative effects of metabolizable energy and amino acid digestibility of corn, soybean meal, and wheat red dog for White Pekin ducks. J Anim Sci 2002;80:3222–9.
Institute of Animal Sciences of CAAS. China feed-database information network centre, state key laboratory of animal nutrition. Tables of feed composition and nutritive values in China. China Feed 2019;21:111–6.
Kaps M, Lamberson WR. Biostatistics for animal science. 3rd ed. Wallingford, CT: CABI Publishing; 2017.
Karunaratne ND, Abbott DA, Hucl PJ, Chibbar RN, Posniak CJ, Classen HL. Starch digestibility and apparent metabolizable energy of western Canadian wheat market classes in broiler chickens. Poultry Sci 2018;97:2818–28.
King D, Ragland D, Adeola O. Apparent and true metabolizable energy values of feedstuffs for ducks. Poultry Sci 1997;76:1418–23.
Losada B, García Rebollar P, Álvarez C, Cachaldora P, Ibánnez MA, Méndez J, et al. The prediction of apparent metabolisable 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 2010;160:62–72.
Losada B, García Rebollar P, Cachaldora P, Álvarez C, Méndez J, De Blas JC. 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. Spanish J Agric Res 2009;7:813–23.
Osho SO, Babatunde OO, Adeola O. Additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dried grains with solubles in mixed diets fed to broiler chickens. Poultry Sci 2019;98:7170–9.
Ragland D, King D, Adeola O. Determination of metabolizable energy contents of feed ingredients for ducks. Poultry Sci 1997;76:1287–91.
Regmi PR, Ferguson NS, Zijlstra RT. In vitro digestibility techniques to predict apparent total tract energy digestibility of wheat in grower pigs. J Anim Sci 2009;87:3620–9.
Sibulde IR. A test of the additivity of true metabolizable energy values of feeding-stuffs. Poultry Sci 1977;56:363–6.
Solt, Castilejo S, López-Vergé S, Casa 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.
The Ministry of Agriculture and Rural Affairs of the People’s Republic of China. The ministry of agriculture and rural affairs of the people’s Republic of China. Nutrient requirements of meat-type duck (NY/T 2122–2012). China: Agri Stand Beijing; 2012.
Valdes IV, Lesoon S. Measurement of metabolizable energy in poultry feeds by an in vitro system. Poultry Sci 1992;71:1493–303.
Wei J, Xie M, Tang J, Wu YB, Zhang Q, Hou SS. The correlation between general nutritive component, metabolizable energy and enzyme hydrolysate gross energy of corn DDGS for ducks. Acta Vet Zootech Sin 2019;50:2032–40.
Wei J, Xie M, Tang J, Wu YB, Zhang Q, Hou SS. The feasibility of enzyme hydrolysate gross energy for formulating duck feeds. Poultry Sci 2020;99:3941–7.
Woyengo TA, Kiarie E, Nyachoti CM. Metabolizable energy and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. Poultry Sci 2010;89:1182–9.
Woyengo TA, Jia R, Beltranena E, Zijlstra RT. In vitro digestion and fermentation characteristics of canola co-products simulate their digestion in the pig intestine. Animal 2016;10:911–8.
Yegani M, Swift ML, Zijlstra RT, Korver DR. Prediction of energetic value of wheat and triticale in broiler chicks: a chick bioassay and an in vitro digestibility technique. Anim Feed Sci Technol 2013;183:40–50.
Zhao J, Zhao F, Zhang H, Tian Z, Wang YM, Yang X, et al. Validation of in vitro digestion using simulated small intestinal fluid with specific digestive activity to predict the metabolizable energy of feed ingredients for duck. Poultry Sci 2019;98:1280–7.
Zhao J, Zhang L, Mi BM, Zhang HF, Hou SS, Zhang ZY. Using a computer-controlled simulated digestion system to predict the energetic value of corn for ducks. Poultry Sci 2014;93:1410–20.
Zhao JT, Zhao F, Zhang HF, Lu QP. Comparison of direct method and difference method in determining metabolizable energy of soybean meal in ducks. Chin J Anim Nutr 2008;20:242–8.