The feasibility of enzyme hydrolysate gross energy for formulating duck feeds

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ABSTRACT Two experiments were designed to investigate the feasibility of enzyme hydrolysate gross energy (**EHGE**) for formulating duck feeds. In experiment 1, six mixed diets and 6 experimental diets (compound feeds) with 20% CP were formulated, and their EHGE, AME, and TME were determined so as to analyze the correlation between EHGE and AME, TME. In experiment 2, six experimental diets with different EHGE levels were further arranged to determine the EHGE requirement for Pekin ducks from hatch to 21 D of age. A total of 384 freshly hatched ducklings was randomly divided into 6 experimental treatments, each treatment containing 8 replicates with 8 ducks per replicate. The results showed that there were a linear correlation between EHGE and AME (r = 0.998, $P < 0.01$), TME (r = 0.997, $P < 0.01$) for 6 mixed diets, and the regression models were AME = 0.996 × EHGE−1.062 (R^2 = 0.996, $P < 0.01$), TME = 0.997 × EHGE+0.304 (R^2 = 0.995, $P < 0.01$). For the 6 experimental diets, EHGE was also positively correlated with AME (r = 0.983, $P < 0.01$), TME (r = 0.984, $P < 0.01$), and the regression models were AME = 1.2054 × EHGE−3.180 (R^2 = 0.967, $P < 0.01$), TME = 1.2054 × EHGE−1.783 (R^2 = 0.967, $P < 0.01$). According to the broken-line model and optimal BW, the EHGE requirement for ducks from hatch to 21 D of age was 2,937 kcal/kg (*calculated value*), 3,182 kcal/kg (*determined value*). In conclusion, EHGE could be used to predict the AME and TME values for mixed diets and compound feeds based on established regression models, and the simulated digestion method in vitro has the potential for effective energy evaluation and formulation for duck feeds.

Key words: duck feeds, enzyme hydrolysate gross energy, feasibility, correlation, requirement

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INTRODUCTION

China is the largest producer of ducks in the world and over 3 billion ducks are raised annually. The feed consumption of ducks is over 35 million tons each year, while feed costs account for 60 to 70% of the total cost of breeding. Therefore, precise evaluation of nutrient values and feed formulation is extremely critical.

Energy is the most important parameter in feed formulation (Moehn et al., 2005). Currently, ME determined by metabolism trial in vivo has been accepted and widely used to formulate poultry feeds. However, the process of ME determination is time consuming, highly expensive, and requires plenty of birds (Boisen and Eggum, 1991; Huang et al., 2003; Kong and Adeola, 2014), and it is easily affected by many factors from animal physiology and external environments (Fang et al., 2012).

To improve the efficiency and precision of the effective energy determination and feeds formulation, several digestion methods in vitro have been developed (Gauthier et al., 1982; Valdes and Leeson, 1992; Boisen and Fernández, 1997; Fang et al., 2012). One of these in vitro methods is a simulated digestion method, which has been established combining enzymology, digestive physiology, and endocrinology (Zhang et al., 2007; Zhao et al., 2014a). It could simulate digestive physiological conditions of pigs, chicks, and ducks, and has the advantages of being less time consuming, lower in cost, and has high precision in enzyme hydrolysate gross energy (**EHGE**) determination (Gauthier et al., 1982; Zhao et al., 2014b; Wang et al., 2016).

In order to apply EHGE to actual livestock production, a good correlation between in vitro and in vivo

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digestion methods is of significant importance (Boisen and Eggum, 1991; Zhao et al., 2014b). Some studies indicated that there was a good relationship between AME and EHGE of corn and soybean meal for ducks (Zhao et al., 2014b; Wei et al., 2019a). However, the feeds consumed by poultry are compound feeds formulated by several feed ingredients. And if companies producing feeds and raising livestocks can develop several regression models based on specific categories of compound feeds formulated by feed ingredients that are usually adopted, the compound feeds may be formulated more accurately and efficiently because of less calculations involving feed ingredients compared to compound feeds and real energy errors in single feedstuffs. Thus, there is a need for further testing the correlation between ME and EHGE of mixed diets and compound feeds.

Considering the advantages of simulated digestion methods in vitro, and previous studies that mainly proved the correlation between ME and EHGE of single feedstuffs, this study was conducted to investigate the correlation between EHGE and ME of mixed diets and compound feeds, and the EHGE requirement for starter Pekin ducks from hatch to 21 D of age to explore the feasibility of EHGE for formulating duck feeds.

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.

Feed Ingredients and Diets

Six mixed diets with 20% CP were formulated using corn, corn gluten meal, soybean meal, and wheat bran with different proportions (Table 1). Six experimental diets were arranged to determine the EHGE requirement for starter Pekin ducks. Except for EHGE levels, these 6 experimental diets met the nutrient requirements of meat-type ducks (Ministry of Agricultural of the People's Republic of China, 2012), which is a standard criterion to guide the feeding of ducks in China. According to the EHGE values of Xiong et al. (2017), the EHGE levels of 6 experimental diets were 2,600, 2,720, 2,840, 2,960, 3,080, and 3,200 kcal/kg (table 2).

The EHGE, AME, and TME values of all diets were determined and the correlation between EHGE and AME, TME were analyzed.

Determination of AME and TME

A total of 84 healthy adult male Pekin ducks were randomly divided into 7 treatments, with 10 adult ducks in each treatment. To ensure consistency in the collecting time of excreta, 2 batches of metabolism trial were conducted. The first batch of metabolism trial included 6 mixed diets and an endogenous energy loss determination, and the other batch involved 6 experimental diets and the endogenous energy loss determination. The endogenous energy loss value was the average of 2 batches.

For the metabolism trial, all ducks were housed in two-tier stainless-steel and individual cages (0.6 × 0.45 × 0.45 m) equipped with a feeder and a nipple drinker. The metabolism room was kept air fresh and had continuous light at around 25°C. The whole determination process was conducted according to the ME determination method of Sibbald (1976) and Ragland et al. (1997). Diets (60 g) were force-fed to each duck in the corresponding treatments and the excreta of ducks were collected for 36 h, respectively. After the whole excreta collection, they were dried at 65°C for about 72 h, measured, and stored until further use to determine DM and GE and calculate AME and TME.

Determination of EHGE

All feed ingredients, 6 mixed diets and 6 experimental diets, used for evaluating the EHGE requirement were prepared to determine EHGE by simulated digestion method, and the method was optimized (Zhao et al., 2014a,b) to simulate gastric, the anterior and posterior branches of the small intestine, digestion for ducks.

For EHGE determination, each sample was ground through a 60-mesh screen and finely mixed. They were added to 5 dialysis bags as 5 replicates within digestion chambers that contained 2 g of grain or 1 g of

Table 1. Nutrient levels and compositions of mixed diets (air-dry basis).

				Proportions of mixed diets			
Samples	DM (%)	GE (MJ/kg)	CP (%)	Corn (%)	Soybean meal (%)	Wheat bran (%)	Corn gluten meal (%)
Corn	88.28	16.51	9.57	100	-	-	-
Soybean meal	90.25	17.86	43.03	-	100	-	-
Wheat bran	90.03	17.09	16.22	-	-	100	-
Corn gluten meal	91.78	21.66	59.52	-	-	-	100
Mixed diet 1	92.59	18.41	21.12	67.83	2.00	12.29	17.88
Mixed diet 2	92.53	18.17	21.21	56.53	4.00	24.57	14.9
Mixed diet 3	92.98	18.14	21.48	45.22	6.00	36.86	11.92
Mixed diet 4	92.94	18.04	20.92	33.92	8.00	49.14	8.94
Mixed diet 5	92.42	17.77	21.22	22.61	10.00	61.43	5.96
Mixed diet 6	92.38	17.67	20.83	11.31	12.00	73.71	2.98

Abbreviation: GE, gross energy.

Table 2. Nutrient levels and composition of experimental diets (air-dry basis).

	EHGE levels (kcal/kg)					
Item	2,600	2,720	2,840	2,960	3,080	3,200
Ingredients (%)						
Corn	51.20	55.74	56.50	60.00	63.20	65.60
Soybean meal	22.30	19.58	19.15	18.46	17.82	16.65
Corn gluten meal	8.00	9.30	9.50	9.50	9.50	10.00
Soybean oil	-	-	1.19	1.53	1.96	2.64
Rice hull	13.56	10.34	8.61	5.45	2.45	-
Dicalcium phosphate	1.73	1.72	1.72	1.70	1.69	1.68
Limestone	1.29	1.32	1.32	1.33	1.35	1.36
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient levels						
EHGE (kcal/kg)[2,3]	2,600	2,720	2,840	2,960	3,080	3,200
CP[4]	20.66	20.51	20.49	21.00	20.56	19.96
Crude fat[4]	1.31	1.42	2.55	2.93	3.66	4.43
Crude fiber[4]	9.14	7.18	6.57	5.11	3.64	2.59
Calcium[3]	0.90	0.90	0.90	0.90	0.90	0.90
Non-phytate phosphorus[3]	0.42	0.42	0.42	0.42	0.42	0.42
Methionine[3]	0.49	0.49	0.49	0.49	0.49	0.49
Lysine[3]	1.10	1.10	1.10	1.10	1.10	1.10
Threonine[3]	0.75	0.75	0.75	0.75	0.75	0.75
Tryptophan[3]	0.22	0.22	0.22	0.22	0.22	0.22

Abbreviation: EHGE, enzyme hydrolysate gross energy.
[1]Supplied by premix per kilogram of total diet: Cu 10 mg, Fe 60 mg, Zn 60 mg, Mn 80 mg, Se 0.3 mg, I 0.2 mg, choline chloride 1,000 mg, riboflavin 8 mg, VA 10 000 IU, VB_6 4 mg, VB_{12} 0.02 mg, VD_3 3,000 IU, VE 20 IU, VK_3 2 mg, folic acid 1 mg, thiamin 2 mg, pantothenic acid 20 mg, biotin 0.2 mg.
[2]The EHGE and AME values of feed ingredients were calculated according to Xiong et al. (2017).
[3]Calculated values.
[4]Determined values.

non-grain sample and 20 mL of simulated gastric fluid containing pepsin (porcine; No. P7000; Sigma, St. Louis, MO). After 4 h of simulated gastric digestion procedure, 10 mL of mixed multienzyme fluid containing trypsin (0785; Amresco, Solon, OH), chymotrypsin (0164; Amresco), and amylase (A3306; Sigma) was individually added and pumped into 5 digestion chambers, 2 mL per chamber, for the anterior and posterior regions of small intestine digestion for 7.5 h, respectively. All of the simulated digestion procedures were performed in a shaking incubator within SDSII (Hunan Zhongben Intelligent Technology Development Co. Ltd., Changsha, China) to ensure each sample and digestive fluid mix completely, and kept at 42°C to simulate dynamic digestion for ducks. After the whole digestive process, all the procedures of determining EHGE were the same as that of ME.

Determination of EHGE Requirement

A total of 384 male White Pekin ducks from hatch were prepared and divided into 6 experimental treatments, each treatment containing 8 replicates with 8 ducks per replicate. They were raised in wire-floor pens from hatch to 21 D of age and had access to water and experimental diets throughout November. There was continuous light, and the temperature was kept at about 28°C from 1 to 3 D and decreased gradually to room temperature until 21 D of age.

At the age of 21 D, ducks of each pen were measured for their average BW, ADG, ADFI, and feed intake:weight gain (feed:gain). Both ADFI and feed:gain were rectified based on mortality. After depriving feeds for 12 h, all ducks from each pen were measured and 2 ducks with similar ADG from each pen (n = 8) were selected randomly. All of the selected ducks were slaughtered by neck and eviscerated manually. Breast meat (both pectoralis major and pectoralis minor), leg meat (both thigh and crus), abdominal fat, and liver were measured electronically, and all observations were recorded in percentages relative to corresponding live BW values. Finally, the EHGE requirement would be estimated according to broken-line model. We obtained 2 EHGE requirements: EHGE calculated value and EHGE determined value. Enzyme hydrolysate gross energy calculated value was determined based on the calculation of citing feed ingredients EHGE (cited, 87% DM) in published standard articles (Xiong et al., 2017). Enzyme hydrolysate gross energy determined value was obtained from experimental diets EHGE (determined, 91% DM) measured directly by simulated digestion in vitro.

Chemical Analysis and Calculation

The DM, CP, and gross energy (**GE**) of all feed ingredients and mixed diets were determined. The GE of feedstuffs, mixed diets, their undigested residues from the simulated digestion method, and their excreta from the metabolism trial were determined by an Oxygen Bomb Calorimeter (Parr 6100; Parr, Moline, IL).

The AME and TME of each sample were calculated as follows (Adeola et al., 1997; King et al., 1997):

AME = (EI−EU)/FI

and

TME = AME + (FEL/FI),

where EI is GE intake of the sample, EU is undigested GE of the sample, FI is the feed intake for each bird (60 g × DM), and FEL is the endogenous energy loss.

The EHGE of each sample was calculated as follows (Zhao et al., 2014a,b):

EHGE = ([sample DM weight × sample DM GE] – [defatted residue DM weight × defatted residue DM GE])/sample DM weight.

Statistical Analysis

In experiment 1, the CORR procedure in SAS 9.2 (SAS Institute, Cary, NC) was used to analyze the correlation between EHGE and AME, TME of mixed diets and experimental diets.

In experiment 2, a one-way ANOVA procedure was carried out to compare differences including growth performance and slaughter performance among 6 experimental treatments, and Duncan's multiple comparison was used if there was a significant difference ($P < 0.05$). The broken-line model was applied to determine the EHGE requirement for starter ducks from hatch to 21 D of age, with the broken line represented as follows:

y = l + u (r − x)where y = BW or weight gain, x = EHGE level (MJ/kg), r = EHGE requirement, l = the result at the turning point, and u = the slope of the curve. In this model, y = l when x > r.

RESULTS

EHGE, ME, and Their Correlation of 6 Mixed Diets

The EHGE and ME of 6 mixed diets are shown in Table 3. The CV of EHGE for the 6 mixed diets was all lower than 1.0%, while the CV of AME and TME for the 6 experimental diets was lower than 5% but greater than the CV of EHGE, which indicates that there was better precision by using the simulated digestion method in vitro to determine EHGE of mixed diets than the metabolism trial in vivo.

The AME and TME increased with increasing EHGE, and they ranged from 10.33 to 15.97, 9.21 to 14.88, and 10.59 to 16.26. Linear and positive correlation were found between EHGE and AME, TME (r = 0.998, $P < 0.0001$; r = 0.997, $P < 0.0001$), and the equations were $Y_{AME} = 0.996 \times EHGE - 1.062$ ($R^2 = 0.996$, $P < 0.01$) and $Y_{TME} = 0.997 \times EHGE + 0.304$ ($R^2 = 0.995$, $P < 0.01$).

EHGE, ME, and Their Correlation of 6 Experimental Diets

The EHGE and ME of 6 experimental diets are shown in Table 4. Similar to the 6 mixed diets, the CV of EHGE for the 6 experimental diets was all lower than 1.0%, and the CV of AME and TME for the 6 experimental diets was lower than 5% but greater than the CV of EHGE, which also indicates that there was better precision by using the in vitro method to determine the effective energy than the in vivo method.

The AME and TME also increased with increasing EHGE, and they ranged from 13.06 to 15.76, 12.38 to 15.87, and 13.77 to 17.26. There were also a linear and positive correlation between EHGE and AME, TME (r = 0.983, $P < 0.01$; r = 0.984, $P < 0.01$), and the regression models were $Y_{AME} = 1.2054 \times EHGE - 3.180$ ($R^2 = 0.967$, $P < 0.01$) and $Y_{TME} = 1.2054 \times EHGE - 1.783$ ($R^2 = 0.967$, $P < 0.01$).

EHGE Requirement of Pekin Ducks From Hatch to 21 D of Age

The growth performance is shown in Table 5; the BW and ADG increased ($P < 0.05$) with increasing EHGE levels of 6 experimental diets, while ADFI and feed:gain both decreased ($P < 0.05$) with higher EHGE levels. The slaughter performance is shown in Table 6; the abdominal fat percentage increased with increasing EHGE levels ($P < 0.05$) of 6 experimental diets, while no difference was found in the percentages of breast meat, leg meat, and liver ($P > 0.05$).

The broken-line regression model was used to analyze the EHGE requirement. For EHGE calculated value and the original data of each pen, the EHGE requirement for BW (y = 1,245.5−0.1933 × [2937.3−x], x ≤ 2,937.3, $P = 0.0007$) was 2,937.3 kcal/kg (*calculated value*). However, for the EHGE determined value, the EHGE

Table 3. EHGE, AME, and TME of 6 mixed diets (DM basis).

Mixed diet no.	EHGE[1] (MJ/kg)	AME[1] (MJ/kg)	TME[1] (MJ/kg)	CV_{EHGE}[2] (%)	CV_{AME}[2] (%)	CV_{TME}[2] (%)
1	15.97 ± 0.09	14.88 ± 0.47	16.26 ± 0.47	0.54	3.18	2.91
2	14.90 ± 0.09	13.94 ± 0.47	15.32 ± 0.47	0.57	3.35	3.05
3	13.91 ± 0.07	12.56 ± 0.36	13.93 ± 0.36	0.48	2.85	2.57
4	12.70 ± 0.06	11.5 ± 0.55	12.87 ± 0.55	0.49	4.76	4.25
5	11.32 ± 0.10	10.37 ± 0.46	11.74 ± 0.46	0.87	4.45	3.93
6	10.33 ± 0.06	9.21 ± 0.46	10.59 ± 0.46	0.56	4.98	4.33

Abbreviation: EHGE, enzyme hydrolysate gross energy.
[1]Means ± SD.
[2]CV_{EHGE}: CV of EHGE for 5 replicates; CV_{AME}: CV of AME for 10 replicates; CV_{TME}: CV of TME for 10 replicates.

Table 4. EHGE, AME, and TME of 6 experimental diets (DM basis).

Experimental diet no.	EHGE[1] (MJ/kg)	AME[1] (MJ/kg)	TME[1] (MJ/kg)	CV_{EHGE}[2] (%)	CV_{AME}[2] (%)	CV_{TME}[2] (%)
1	13.06 ± 0.08	12.38 ± 0.39	13.77 ± 0.39	0.65	3.13	2.81
2	13.59 ± 0.09	13.62 ± 0.39	15.01 ± 0.39	0.66	2.88	2.61
3	14.21 ± 0.09	13.73 ± 0.45	15.13 ± 0.45	0.64	3.26	2.96
4	14.64 ± 0.14	14.4 ± 0.45	15.79 ± 0.45	0.98	3.13	2.85
5	15.35 ± 0.1	15.32 ± 0.28	16.71 ± 0.28	0.62	1.83	1.67
6	15.76 ± 0.09	15.87 ± 0.21	17.26 ± 0.21	0.59	1.32	1.21

Abbreviation: EHGE, enzyme hydrolysate gross energy.
[1]Means ± SD.
[2]CV_{EHGE}: CV of EHGE for 5 replicates; CV_{AME}: CV of AME for 10 replicates; CV_{TME}: CV of TME for 10 replicates.

requirement ($y = 1{,}245.5 - 0.1916 \times [3182.2 - x]$, $x \leq 3{,}182.2$, $P = 0.0006$) was approximately 3,182 kcal/kg (*determined value*) for ducks from hatch to 21 D of age.

DISCUSSION

A good correlation between effective energy evaluations by the in vitro method and bioassay method in vivo is necessary to apply the in vitro method in practical purpose (Boisen and Eggum, 1991; Boisen and Fernández, 1997), and some previous studies were carried out mainly to investigate the correlation between the effective energy or digestibility determined by the in vitro method and in vivo method for single feed ingredients. Therefore, we conducted 2 experiments to investigate the correlation for mixed diets and compound feed to support previous studies in the correlation of single feed ingredients and explore the EHGE requirement. In our study, experiment 1 was arranged first to mainly investigate the relationship between EHGE and AME, TME of mixed diets and compound feeds for ducks. Experiment 2 was conducted after confirming the good correlation between EHGE and ME to explore the EHGE requirement and compare the difference between the determined value and calculated value of EHGE requirement for ducks from hatch to 21 D of age.

In experiment 1, six mixed diets were formulated with 4 common feed ingredients and 6 compound feeds were formulated with different EHGE levels based on defined proportions. Linear and positive correlation was found between EHGE and AME, TME of 6 mixed diets ($r = 0.998$, $P < 0.01$; $r = 0.997$, $P < 0.01$) and 6 compound feeds ($r = 0.983$, $P < 0.01$; $r = 0.984$, $P < 0.01$) for ducks. Our results were consistent with those of Zhao et al., 2014a,b. In their study, in vitro digestible energy (**IVDE**, equivalent to EHGE) was highly and positively correlated with AME and TME of 30-corn feed for ducks ($r = 0.9419$, $P < 0.01$; $r = 0.9403$, $P < 0.01$). Another study (Zhao et al., 2014a,b) showed a linear relationship between IVDE and AME, TME of 16 feed ingredients for roosters ($R^2 = 0.97$, $P < 0.01$; $R^2 = 0.9403$, $P < 0.01$). Yegani et al. (2013) found that in vitro AME (equivalent to EHGE) was also correlated with AME_n of 8 feedstuffs for broiler chicks ($R^2 = 0.81$, $P < 0.01$). From our recent results (Wei et al., 2019a,b), we found a remarkably linear correlation between EHGE and AME, TME of soybean meal ($r = 0.976$, $P < 0.01$; $r = 0.998$, $P < 0.01$), and corn distillers dried grains with solubles for ducks ($r = 0.995$, $P < 0.01$; $r = 0.996$, $P < 0.01$). In addition, some correlation between in vitro and in vivo digestibility of feed ingredients was also found. Boisen and Fernández (1997) indicated that there was a close linear relationship between in vitro and in vivo total tract energy digestibility of 90 feedstuffs for pigs ($R^2 = 0.94$). Huang et al. (2003) and Regmi et al. (2008) found that in vitro energy digestibility was correlated with in vivo energy digestibility of barley for pigs ($R^2 = 0.88$; $R^2 = 0.97$). The fact that needs to be emphasized is that the linear equations R^2 using different kinds of feed ingredients were less consistent than using 1 designated category of feedstuffs in in vivo and in vitro

Table 5. Effect of dietary EHGE content on growth performance of Pekin ducks from hatch to 21 D of age.

EHGE[1] (calculated value, 87% DM) (kcal/kg)	EHGE[2] (determined value, 91% DM) (kcal/kg)	BW[3] (g/bird per D)	ADG[3] (g/bird per D)	ADFI[3] (g/bird per D)	Feed:gain[3] (g:g)
2,600	2,841	1,191 ± 29[b]	57±1[b]	109±7[a]	1.92[a]
2,720	2,956	1,181 ± 44[b]	56±2[b]	106±4[a,b]	1.88[a,b]
2,840	3,092	1,238 ± 45[a]	59±2[a]	108±3[a]	1.83[b]
2,960	3,184	1,235 ± 55[a]	59±3[a]	103±3[b]	1.75[c]
3,080	3,338	1,254 ± 33[a]	60±2[a]	102±4[b,c]	1.7[c,d]
3,200	3,428	1,248 ± 36[a]	60±2[a]	98±3[c]	1.64[d]
P-value		0.0027	0.0026	<0.0001	<0.0001

[a–c]Means with different superscripts within the same column differ significantly ($P < 0.05$).
Abbreviation: EHGE, enzyme hydrolysate gross energy.
[1]Calculated value is calculated according to Xiong et al. (2017).
[2]Determined value is measured.
[3]Results are means with n = 8 per group.

Table 6. Effect of dietary EHGE content on slaughter performance of Pekin ducks from hatch to 21 D of age.[1]

EHGE (calculated value, 87% DM) (kcal/kg)	EHGE (determined value, 91% DM) (kcal/kg)	Breast meat[2] (%)	Leg meat[2] (%)	Abdominal fat[2] (%)	Liver[2] (%)
2,600	2,841	2.1 ± 0.3	9.97 ± 0.7	0.83 ± 0.2[c]	2.88 ± 0.2
2,720	2,956	2 ± 0.4	9.66 ± 0.8	0.84 ± 0.2[c]	2.93 ± 0.3
2,840	3,092	2 ± 0.2	9.5 ± 0.7	0.95 ± 0.3[b,c]	3 ± 0.4
2,960	3,184	2 ± 0.3	9.39 ± 1.1	0.95 ± 0.3[b,c]	2.9 ± 0.4
3,080	3,338	2.2 ± 0.5	9.59 ± 1.1	1.02 ± 0.2[b]	2.94 ± 0.6
3,200	3,428	2 ± 0.3	9.34 ± 0.6	1.2 ± 0.2[a]	2.72 ± 0.1
P-value		0.7087	0.3226	<0.0001	0.4103

[a–c]Means with different superscripts within the same column differ significantly ($P < 0.05$).
Abbreviation: EHGE, enzyme hydrolysate gross energy.
[1]All values were in percentages relative to live BW.
[2]Results are means with n = 8 per group.

methods (Boisen and Fernández, 1997). A regression model or an equation is not universally used; so it is extremely important to develop mathematic models for specific feed ingredients and compound feeds, which are more comprehensive to predict ME by EHGE and reduce errors during calculations from feed ingredients to compound feeds.

In experiment 2, six experimental diets in experiment 1 with different EHGE levels were adopted further to estimate the EHGE requirement for Pekin ducks from hatch to 21 D of age. In our study, the BW and ADG increased ($P < 0.05$), while ADFI and feed:gain both decreased ($P < 0.05$) with rising EHGE levels of 6 experimental diets, in agreement with the observations of Scott et al. (1959), Wilson (1975), and Fan et al. (2008). Also, in experiment 2, the abdominal fat percentage increased with rising EHGE levels ($P < 0.05$), while no difference was found in the percentages of breast meat, leg meat, and liver ($P > 0.05$). Zhao et al. (2009) and Xie et al. (2010) showed similar results on ducks, and Jackson et al. (1982) and Dozier et al. (2006) on broilers. However, the results of previous studies mainly focused on the AME requirement and the EHGE requirement has not been estimated and reported so far. Fan et al. (2008) and Xie et al. (2010) found that the AME requirements for 2 to 6-week-old and 0 to 21-day-old ducks were 3,008 and 3,019 kcal/kg, respectively. In our study, the EHGE requirements were 2,937 kcal/kg (*calculated value*) and 3,182 kcal/kg (*determined value*).

In experiment 1, for 6 mixed diets and 6 compound feeds, the CV of AME, TME ranged from 2.85 to 4.98 and 2.57 to 4.33, 1.32 to 3.26 and 1.21 to 2.96, respectively, while the CV of EHGE for mixed diets or experimental diets was all within 1.0%, which was also consistent with the results of Zhao et al. (2014a). In their study, the CV of IVDE ranged from 0.33 to 0.54 for wheat, and from 0.2 to 1.14 for corn. Therefore, the simulated digestion method in vitro showed higher precision than the metabolism trial in vivo. And in experiment 2, we found that the EHGE *determined* value requirement (3,182 kcal/kg) was higher than the *calculated* value (2,937 kcal/kg). There are some specific reasons for this result. Irrespective of the AME requirement or EHGE requirement, when we make a feeds formulation, the energy value adopted for the feed ingredient is the average of many feed ingredient samples cited in published standards or papers. In addition, the real energy value of a feedstuff would vary because of different origins or batches. In order to obtain an accurate energy value for feed formulation, we have to determine the real energy of feedstuffs or compound feeds. Therefore, the simulated digestion method in vitro is a promising method and EHGE has the potential to be applied to feed formulation for ducks.

In conclusion, the AME and TME can be predicted by EHGE including feed ingredients and mixed diets, and the EHGE requirement is 3,182 kcal/kg for Pekin ducks from hatch to 21 D of age. EHGE determined by the simulated digestion method in vitro is a promising approach that can be applied to feed formulation for ducks.

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