

## Correlation between chemical composition, EHGE and TME of corn for ducks

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### Abstract

Correlations between chemical composition, enzymatic hydrolysate gross energy (EHGE), and true metabolizable energy (TME) of corn for ducks were investigated. Twenty-two corn samples were collected from various regions in 11 provinces of China. The crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), Ash, gross energy (GE), dry matter (DM), amylopectin (AP), amylose (AM), total starch (TS), and AP/AM were determined for each sample. Five of the samples of corn were chosen at the mean, mean  $\pm$  1 standard deviation (SD), and mean  $\pm$  2 SD based on AP/AM. The EHGE of these samples was analysed using the pepsin-artificial small intestinal fluid enzymatic method. These five samples were also force-fed to male Cherry Valley ducks to assay their TME. Finally, correlation analyses were performed, and regression equations were established. Ash content, GE, and TS were highly related to EHGE. Univariate prediction equations were  $\text{EHGE} = 11.8566\text{Ash}^{-0.0421}$  ( $P < 0.05$ ),  $\text{EHGE} = 0.1535\text{GE}^{1.5642}$  ( $P < 0.05$ ), and  $\text{EHGE} = 0.1020\text{TS}^{1.1561}$  ( $P < 0.05$ ). The total starch, AP/AM, and ash of the chemical compositions were highly related to TME. The corresponding univariate regression equations were  $\text{TME} = 21.9355\text{TS}^{-0.0910}$  ( $P < 0.05$ ),  $\text{TME} = 15.6590\text{AP/AM}^{-0.0559}$  ( $P < 0.05$ ), and  $\text{TME} = 15.0778\text{Ash}^{0.0442}$  ( $P < 0.05$ ). The mean EHGE was equivalent to 78.5% of TME, but their correlation coefficient was low. In conclusion, chemical composition was predictive of EHGE and TME of corn samples for ducks, but the correlation of EHGE and TME was low.

**Keywords:** Cherry Valley duck, amylopectin, amylose, true metabolizable energy

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### Introduction

Duck production and breeding are important at present (Bond *et al.*, 2008; Lin *et al.*, 2014). Corn is the commonest grain feedstuff and is used widely in duck production. It is rich in starch, which is a direct and effective material to supply energy (Theurer *et al.*, 1999). So, accurate determination of the energy utilization efficiency of corn was the main basis for deciding to establish a standard of nutritional requirements and to optimize feed formulation. Varieties of corn have different chemical compositions because of diverse sources and processing techniques. True metabolizable energy (TME) values differ in plant species (Dugger *et al.*, 2007). So it is important to establish a database of types of corn. Knowledge of the ME of corn for ducks (Chinese Feedstuff Database, 2017) is limited. More ME values are needed of varieties of corn for ducks in China. Traditional biological methods contained feeding and metabolic tests to determine the energy of feedstuffs and the digestibility of nutrients. These methods were visual and scientific, but they were time consuming, expensive, and difficult to standardize. Thus, it was crucial improve the determination of TME.

Neutral detergent fibre (NDF) can be an effective indicator of the TME of wheat and wheat by-products for ducks (Wan *et al.*, 2009; Mazhari *et al.*, 2011). These results suggest strongly that the chemical composition of a feedstuff may be used to predict TME. Methods to determine chemical composition have been perfected by the Association of Official Analytical Chemists (AOAC, 2000). The enzymatic method is a dynamic digestion process, which adds exogenous enzymes to feedstuff, and is easy and rapid to implement. Digestibility of organic matter and energy content of hay predicted by the rumen fluid-neutral detergent method and the cellulose method had similar levels of accuracy (Iantcheva *et al.*, 1999). Thus, the

enzymatic method can be used to predict energy value. However, there is little information about the correlation between chemical composition, EHGE and TME of corn for ducks from China.

Objectives of this study were to i) determine the chemical composition of 22 corn samples and select five representative corn samples based on the amylopectin to amylose ratio (AP/AM); ii) investigate the correlations between chemical composition, EHGE, and TME of these samples; and iii) establish relatively accurate prediction models to estimate TME.

## Materials and Methods

Twenty-two corn samples were collected from different regions of China (Table 1). The amount of material in each sample was reduced by coning and quartering, and the remaining subsamples were ground through 40-mesh screen. Chemical composition of each ground sample (Table 2) was determined by methods from AOAC (2000) for dry matter (DM) (method 934.01), Ash (method 942.05), crude protein (CP) (method 955.04), and ether extract (EE) (method 920.39). Neutral detergent fibre was determined according to Van Soest *et al.* (1991) and gross energy (GE) was determined by adiabatic calorimetry (Parr Instruments Co., Moline, IL). Total starch, AM, and AP were determined using a modification of the Sene *et al.* (1997) method whereby corn was passed through a 100 mesh screen. Because the AP/AM ratio is an indicator of the structure of corn starch and affects starch digestion, it was used to choose five representative corn samples for further analysis.

**Table 1** Sources of 22 samples used to assess nutritional value of corn for ducks

Sample	Source	Region	Sample	Source	Region
1	Yantai in Shandong	East China	12	Yicheng in Hubei	Central China
2	Haimen in Jiangsu	East China	13	Xuchang in Henan	Central China
3	Hefei in Anhui	East China	14	Chengchow in Henan	Central China
4	Wuhan in Hubei	Central China	15	Hohhot in Inner Mongolia	North China
5	Xiantao in Hubei	Central China	16	Chifeng in Inner Mongolia	North China
6	Hanchuan in Hubei	Central China	17	Chengde in Hebei	North China
7	Xiangfan in Hubei	Central China	18	Shijiazhuang in Hebei	North China
8	Honghu in Hubei	Central China	19	Shenyang in Liaoning	Northeast China
9	Hannan in Hubei	Central China	20	Changchun in Jilin	Northeast China
10	Yichang in Hubei	Central China	21	Qiqihar in Heilongjiang	Northeast China
11	Xianning in Hubei	Central China	22	Urumqi in Sinkiang	Northwest China

Three 0.5 g samples of each of these corn samples were placed in 150 ml conical flasks for *in vitro* digestion. A volume of 10 mL pepsin hydrochloric acid solution with concentration of 2 g/L was added to each flask. The concentration of hydrochloric acid was calibrated with anhydrous sodium carbonate as 0.075 mol/L. Each flask was then incubated in a water bath at 38 °C for three hours. The solution was neutralized with 0.75 mL 1 mol/L sodium hydroxide solution, and 10 mL artificial small intestinal fluid was added to each flask. The pH in each flask was approximately 6.60. The artificial small intestinal fluid was composed of amylase, lipase, trypsin, and chymotrypsin. The flask was returned to the water bath and incubated at 38 °C for an additional 22 hours. The suspension was suction filtered, and the filter dried at 105 °C and weighed. Gross energy and DM were determined for the residue (R). Following Zhao (2014), enzymatic dry matter digestibility (EDMD) and EHGE were calculated as follows:

$$EDMD = \frac{100(F \times DM_F - R)}{F \times DM_F}$$

$$EHGE = \frac{100(F \times DM_F \times GE_F - R \times GE_R)}{F \times DM_F}$$

where: F = weight of feedstuff;  $DM_F$  = DM of the feedstuff (%); R = weight of dry residue after *in vitro* digestion;  $GE_F$  = GE of the feedstuff (KJ/g); and  $GE_R$  = GE of the residue after *in vitro* digestion (KJ/g).

**Table 2** Chemical composition of 22 corn samples from across China expressed on a dry matter basis

Sample	AP, %	AM, %	TS, %	AP/AM	CP, %	EE, %	NDF, %	Ash, %	DM, %	GE (MJ/kg)
1	27.10	14.31	66.79	1.89	6.88	3.14	11.47	2.18	86.52	15.50
2	28.65	15.28	62.39	1.88	7.48	2.34	11.82	1.64	87.56	16.19
3	28.44	12.55	60.24	2.27	8.00	2.80	11.02	1.36	85.49	16.16
4	25.58	12.90	64.31	1.98	7.44	4.16	11.49	1.49	85.61	15.75
5	26.74	14.37	58.68	1.86	6.76	3.07	12.31	2.09	85.64	15.76
6	27.71	12.05	56.60	2.30	6.54	3.48	10.77	0.94	85.11	15.56
7	31.23	11.76	61.73	2.66	6.69	3.80	11.02	1.04	85.36	16.64
8	29.70	12.76	63.26	2.33	7.26	2.64	9.83	1.42	84.71	15.48
9	19.61	10.37	56.02	1.89	8.03	5.29	11.19	0.91	85.27	16.33
10	31.94	12.94	56.00	2.47	7.88	4.10	11.32	1.22	85.68	15.23
11	27.23	10.45	57.43	2.60	8.30	4.83	12.08	1.22	87.00	16.58
12	32.05	13.03	60.49	2.46	7.52	6.00	10.85	1.24	87.74	16.48
13	30.42	14.30	62.75	2.13	7.32	3.23	10.42	1.01	86.45	15.66
14	28.85	12.77	53.60	2.26	8.76	2.02	10.01	2.37	84.31	15.28
15	23.41	14.15	66.67	1.65	5.81	4.33	12.33	0.81	87.66	16.69
16	24.84	15.28	66.15	1.63	6.32	3.45	12.22	1.00	86.20	15.77
17	27.45	14.19	61.62	1.93	8.23	4.94	12.41	1.11	88.21	16.40
18	20.59	13.12	61.50	1.57	7.37	4.05	15.95	2.75	87.13	16.37
19	27.00	14.05	63.69	1.92	8.40	3.68	10.27	1.06	84.69	15.25
20	24.13	14.41	64.89	1.67	6.93	4.46	10.59	1.18	86.58	17.10
21	20.75	14.39	61.22	1.44	6.78	3.66	14.25	1.11	86.74	16.29
22	19.31	12.43	56.00	1.55	7.97	4.32	15.93	1.38	87.95	16.74
Mean	26.49	13.27	61.00	2.02	7.39	3.81	11.80	1.39	86.26	16.05
SD	3.77	1.32	3.72	0.35	0.74	0.95	1.63	0.50	1.12	0.54

AP: amylopectin, AM: amylose, AP/AM: ratio of amylopectin to amylose; TS: total starch, CP: crude protein, EE: ether extract, NDF: neutral detergent fibre, GE: gross energy, DM: dry matter.

The protocol of the animal experiment was approved by the Animal Experimental Ethical Inspection of Laboratory Animal Centre, Huazhong Agriculture University (HZAUDU-2015-005). Forty-eight adult male Cherry Valley ducks were selected from Chunjiang duck factory (Huangpi, Hubei Province, China). The ducks were in good health and were similar in weight ( $3.24 \pm 0.36$  kg). Prior to the experiment there was a seven-day wash-out period. The ducks were randomly assigned to six treatments of eight replicates of one duck each. Five groups of ducks were force-fed with one of the five corn samples, and the sixth group was fasted to determine the loss of endogenous energy. Ducks were caged individually throughout the experiment.

Force-feeding was carried out according to the modified bioassay of Sibbald (1976) and McNab and Blair (1988). Briefly, the complete formula diet (Wuhan Chia Tai Co., Ltd.) for adult meat ducks was removed on the first day of experiment. At eight and 32 hours after feed withdrawal, each duck was force-fed with a warm 38.5% (v/v) glucose solution to reduce the stress of fasting. Then, at 36 hours after feed withdrawal, 60 g representative corn samples (Samples 6, 11, 15, 19, 21) varying in the ratio of AP to AM were force-fed to the fasted ducks. Each duck had a clean tray inserted beneath its cage and the excreta were collected for 36 hours. Samples were deemed valid if the duck did not vomit and the excreta was not lost. There were at least six excreta samples for each tested feed sample. To determine the loss of endogenous energy, ducks were fasted for 72 hours, and excreta samples were collected for 36 hours. A volume of 10 ml of 10% hydrochloric acid was added to the excreta of each duck during the collecting period. After drying at 65 °C and weighing, the excreta samples were ground through a 40-mesh screen and the GE and DM were determined. The apparent metabolizable energy (AME) and TME were calculated according to Wan (2009):

$$TME = [EI - (EO - EEL)]/FI \text{ and}$$

$$AME = (EI - EO)/FI$$

where: EI = GE of the intake of the feedstuff; EO = GE voided of the feedstuff; EEL = loss of endogenous energy; and FI = intake of the feedstuff (60 g).

The SAS statistical analysis software (SAS Institute Inc., Cary, North Carolina, USA) was used for all data analyses. Correlation analyses were performed using CORR program. The results were shown as mean  $\pm$  SD. Regression equations were established between chemical composition and EHGE, and chemical composition and TME. Enzymatic hydrolysate gross energy and TME were compared by T-test. The variance was considered significant when  $P < 0.05$ .

## Results

The EHGE and EDMD of the five representative samples are shown in Table 3. On a dry matter basis, the EHGE was from 9.50 to 16.77 MJ/kg, and EDMD was between 58.73 and 90.12. The TME of the corn samples is shown in Table 4. On air dry matter basis, TME varied from 13.68 MJ/kg to 15.91 MJ/kg.

**Table 3** Nutritional values of 5 representative corn samples determined by the enzymatic method (% dry matter basis)

Nutritional values	Sample					Mean	SD
	21	15	19	6	11		
GE (MJ/kg)	18.78	19.04	18.01	18.28	19.06	18.63	0.42
DM, %	86.74	87.66	84.69	85.11	87.00	86.24	1.14
EDMD, %	90.12	72.91	70.47	69.92	58.73	72.43	10.11
EHGE (MJ/kg)	16.77	12.44	10.44	10.09	9.50	11.85	2.65

GE: gross energy, DM: dry matter, EDMD: enzymatic dry matter digestibility, EHGE: enzymatic hydrolysate gross energy

**Table 4** True metabolizable energy of five representative corn samples (% dry matter basis)

Nutritional values	Sample					Mean	SD
	21	15	19	6	11		
True metabolizable energy, MJ/kg	15.91	15.50	13.68	14.66	15.70	15.09	0.82
Apparent metabolizable energy, MJ/kg	14.13	13.87	12.04	13.02	13.89	13.21	0.68
Digestibility of dry matter, %	86.03	81.85	77.63	83.89	83.09	81.62	2.16

Correlations of chemical composition with EHGE and TME are shown in Table 5. Ash, GE, and TS were highly correlated with EHGE, so three regression equations were established as:  $EHGE = 11.8566Ash^{-0.0421}$ ;  $EHGE = 0.1535GE^{1.5642}$ ; and  $EHGE = 0.1020TS^{1.1561}$  (all  $P = 0.01$ )

Likewise, TS and AP/AM were highly, but negatively correlated to TME. Ash was highly positively correlated to TME. Thus, three additional regression equations were established as:

$$TME = 11.8566Ash^{0.0442} \quad TME = 21.9355TS^{-0.910}; \text{ and } TME = 0.6590 \left( \frac{AP}{AM} \right)^{-0.0559} \quad (\text{all } P < 0.001)$$

The correlation coefficient between EHGE and TME was low at 0.3853. Values of EHGE and TME were compared by T-test and the EHGE values were less than the values of TME ( $P = 0.0436$ ). The mean difference between TME and EHGE was 3.24 MJ/kg and thus EHGE approximately 78.5% of TME.

**Table 5** Correlations of chemical components with enzymatic hydrolysate gross energy and total gross energy

Chemical component	Enzymatic hydrolysate gross energy		Total gross energy	
	Correlation	<i>P</i> -value	Correlation	<i>P</i> -value
Amylopectin	-0.0076	0.9657	-0.2777	0.6069
Amylose	0.2124	0.6738	-0.7958	0.1611
Total starch	0.5821	0.3345	-0.8714	0.1012
Amylopectin:amylose ratio	-0.0657	0.8537	-0.8185	0.1431
Gross energy	0.6181	0.3047	0.0350	0.9050
Crude protein	-0.4534	0.4443	-0.5180	0.9043
Ether extract	0.1927	0.6950	0.3940	0.4973
Ash	-0.8853	0.0902	0.8087	0.1508
Neutral detergent fibre			0.0529	0.8736
Dry matter	0.5335	0.3754	0.0501	0.8782

## Discussion

The observed values for chemical composition of the corn samples showed that the mean content of DM, CP, EE, and ash approached previously published values (NRC, 1994; Zhang *et al.*, 2018). Batal *et al.* (2012) reported that the chemical compositions of yellow corn were that DM was 86%, CP was 7.5%, EE was 3.5%, crude fibre (CF) was 1.9%, ash was 1.1%, and ME for poultry was 14.17 MJ/kg. Most of the chemical compositions of corn were consistent with these results, although CF was not consistent with NDF in this study, because the methods of determination of CF and NDF differ. Furthermore, the results of AME and TME approached the ME for poultry. This suggests that varieties of corn in China are similar to those grown internationally. In this study, the variations of EE, ash and AP/AM were greater than others' indicators of nutritional content because the cultivars and growing conditions were different (Sandhu *et al.*, 2004). Zhou (2010) reported the AM, AP, and AP/AM of 21 corn samples as 19.82%, 60.09%, and 3.04%. The AM value approached that of this study, but the AP and AP/AM were higher than the present results.

The amylopectin to amylose ratio is not only an important index to reflect starch structure, but also a crucial factor in the digestion of starch. In the 22 samples of corn that were evaluated in this study, the ratio was reasonably ( $P \leq 0.10$ ) correlated with NDF (-0.61), DM (-0.37), and TS (-0.35). Therefore, five samples were chosen for further study based on the AP/AM. As the content of AP increased and the content of AM decreased their AP/AM increased from 1.44 to 2.60.

The *in vitro* experiment, which used enzymes to simulate the *in vivo* digestion process, developed quickly. A one-step enzymatic method and a two-stage method were proposed by some researchers (Denek & Deniz, 2004a). The *in vitro* method was successful in predicting digestible energy and digestibility of DM, and organic matter (Fang *et al.*, 2012). Previously, *in vivo* DM, organic matter digestibility, and ME of corn were found to be 83.81%, 83.90% and 13.52 MJ/kg, respectively, with the corresponding *in vitro* values being 81.21%, 82.27%, and 12.90 MJ/kg (Denek & Deniz, 2004b). These authors concluded that the enzyme technique could be used instead of the *in vivo* method. In this study, the mean DM, EDMD, and EHGE of the five samples by the *in vitro* method were 86.24%, 72.43%, and 11.85 MJ/kg. The mean digestibility of dry matter (DDM) and TME by the *in vivo* method were 81.26% and 15.09 MJ/kg. It is suggested that *in vitro* method might be used to determine in duck diet. However, ash, GE, and TS were highly related to EHGE allowing them to be used to predict EHGE effectively.

The AME of corn for ducks in the Chinese Feedstuff Database (2017) was 13.01 MJ/kg, approaching the value of 13.21 MJ/kg found in this study. This suggests that these 22 corn samples are representative of corn in China. In this study, TS, AP/AM, and ash were chosen to predict TME, because these three characteristics of corn that could be assessed chemically were closely related to TME. This was consistent with the results of Ren *et al.* (2012) and Jie *et al.* (2013). Ren *et al.* (2012) used corn starch as a basal diet in determining the TME of protein feedstuffs for Chinese Yellow chickens. Likewise, Jie *et al.* (2013) used the chemical characteristics of corn distillers dried grains with solubles to predict their AME and TME contents for roosters. However, the present results were not completely consistent with those of Zhao (2008), who

reported that NDF and GE could be used to predict TME. These authors used corn from different regions, but Zhao *et al.* (2008) used corn calibration samples. Perai *et al.* (2010) predicted the TME of meat and bone meal based on the CP, EE, and Ash content. Ahmadi *et al.* (2008) also used the group method based on these chemical compositions to predict the TME of feather meal and poultry offal meal. Their results were similar to the results of this study. The correlation coefficients between these chemical compositions and TME were high, but they were lower than TS and AP/AM, suggesting that the content of starch combined with other chemical composition could predict TME.

The correlation coefficient between EHGE and TME was low and the EHGE was significantly less than TME ( $P < 0.05$ ). Zhao (2008) also reported that EHGE was lower than TME by 1.63 - 2.69 MJ/kg, with a mean difference of 2.26 MJ/kg. Differences in the samples of corn used in the two studies may explain the relatively minor differences between the present results and those that were used previously.

## Conclusions

Chemical composition was predictive of EHGE and TME of corn samples for ducks. However, the correlation of values for EHGE and TME was low.

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## Authors' Contributions

YJC, ZYW, and CGD contributed equally to this work, performing the experiments and writing the paper. ZLQ conceived and designed the experiments. YQG, XHS, ZKG, CZ, YFZ, and HMY participated in some aspects of the work, including conducting the experiments and analysing the data. They also reviewed the content of this article and suggested revisions.

## Conflict of Interest Declaration

The authors have declared that no competing interests exist.

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