Metabolizable and net energy values of corn stored for 3 years for laying hens

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ABSTRACT The apparent metabolizable energy (**AME**), AME corrected to zero-nitrogen retention (**AMEn**), and net energy (**NE**) values of 2 corn samples both stored for 3 yr were determined in laying hens with reference diet substitution method. Reference diet was formulated according to standard layer requirement, and test diets contained 50% of corn samples and 50% of the reference diet. Fifty-four Hy-Line Brown hens at the age of 36 wk were used. The heat production and energy metabolism of birds were measured in open-circuit respiratory chambers with 6 replicates (3 birds per replicate) per diet in a randomized design. Birds were fed experimental diets for 7 D in the chamber as adaptation. During the following 3 D, feed intake, metabolizable energy value, nitrogen balance, energy balance, egg production, O_2 consumption, CO_2 production, and energy efficiency were determined. The AME values of corn 1 and corn 2 were 3,485 and 3,675 kcal/kg DM, respectively. The corresponding AMEn values were 3,452 and 3,596 kcal/kg DM, and the NE values were 2,575 and 2,693 kcal/kg DM, respectively. The NE:AME ratios of corn 1 and corn 2 were 74.4 and 73.3%, respectively. The NE:AMEn ratios of corn 1 and corn 2 were 75.0 and 74.9%, respectively. The AME, AMEn, and NE values of the 2 corn samples both stored for 3 yr were lower than the literature values for fresh corn.

Key words: metabolizable energy, net energy, corn, laying hen

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INTRODUCTION

Energy represents at least 60% of total cost in poultry feed. It is important to accurately estimate the available energy content of feed ingredients. The true or apparent metabolizable energy corrected to zero-nitrogen retention (**TMEn**; **AMEn**) is currently used to formulate feed in the poultry industry. However, unlike the net energy (**NE**) system, the ME system fails to take into consideration the energy loss that is resulted from the assimilation of the dietary nutrients. This energy loss is frequently termed the heat increment (**HI**) of digestion (Pirgozliev and Rose, 1999). The HI is usually calculated by subtracting the fasting heat production (**FHP**) from the total heat production (**HP**) of fed animals (Barzegar et al., 2019a). The NE is equal to ME minus HI. It has been reported that the ME system overestimates the NE of high-protein ingredients and underestimates energy-rich feedstuffs (Pirgozliev and Rose, 1999). According to a study reported by Barzegar et al. (2019a), the energy efficiencies of gross energy (**GE**) for AME of ether extract (**EE**), starch, and CP in laying hens were 78, 95, and 60%, while the corresponding NE:AME values were 104, 78, and 49%, respectively. These observations indicate that the NE system would be more accurate for representing the "true" available energy of feed ingredients than the ME system.

Adult cockerels or growing chickens are usually used to estimate the ME values of feed ingredients by the reference diet substitution method. However, these ME values determined in adult or growing broilers may be not completely appropriate for laying hens (Barzegar et al., 2019b). Kaminiska (1979) reported that the AME of a diet in Leghorn chicks was between 1 and 7% higher than that in broiler chicks, and this increased AME may be contributed to increased gizzard weights and longer intestines in Leghorn chicks. Spratt and Leeson (1987) observed that MEn of same diet in regular broiler breeder hens was less than that in Comb White

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Leghorn birds. Cozannet et al. (2010) reported that the AMEn values of wheat dried distillers grains with solubles for roosters, broilers, layers, and turkeys were 2,471, 2,373, 2,302, and 2,165 kcal/kg DM, respectively. These results indicate that the ME values of ingredients vary between types of the animals and physiological stages. In addition, dietary composition may affect the available energy values of feed ingredients. The supplementation of limestone as a source of calcium in the diet of laying hens results in increased endogenous energy losses and decreases the AMEn content of ingredients for laying hens compared to roosters (Cozannet et al., 2010). It is preferable to use the animal-specific energy values of ingredients to formulate the feed. Hence, accurate estimates of the real available energy of feed ingredients according to the animal species, breeds, and ages are necessary.

Corn, a commonly used energy source in the poultry feed industry, is usually stored for certain periods that can be up to several years before being used (Bartov, 1996). The nutritional value of corn may be altered due to the lengthy storage. Pomeranz (1974) noted that storage may decrease the fat and increase the free fatty acids content of grains, which may reduce their energy content. There is a large volume of corns stored by grain reserve companies for 3 or more years in China. However, limited data are available on the energy value of these stored corns in laying hens. Thus, the main objective of the present study was to determine the ME and NE values of 2 corn samples with a low or high kernel density both stored for 3 yr using the reference diet substitution method in laying hens.

Table 1. Composition and kernel density of corn ingredients (%, as is).[1]

Items	Corn 0	Corn 1	Corn 2
Dry matter	86.1 ± 0.11	86.9 ± 0.27	86.6 ± 0.38
Crude protein	8.0 ± 0.17	8.1 ± 0.08	8.2 ± 0.12
Starch	63.2 ± 0.58	64.4 ± 0.54	64.3 ± 0.16
Crude fat	2.6 ± 0.10	3.0 ± 0.12	2.9 ± 0.19
Crude fiber	1.4 ± 0.12	1.4 ± 0.02	1.6 ± 0.06
Ash	1.3 ± 0.13	1.1 ± 0.02	1.1 ± 0.11
ADF	3.8 ± 0.08	3.6 ± 0.21	3.4 ± 0.09
NDF	8.3 ± 0.67	8.2 ± 0.37	8.3 ± 0.52
Gross energy (kcal/kg)	3,891 ± 13.1	3,920 ± 15.1	3,927 ± 18.3
Kernel density (g/L)	723 ± 2.9	696 ± 2.7	730 ± 2.1
Indispensable amino acids			
Arginine	0.30 ± 0.01	0.29 ± 0.01	0.30 ± 0.01
Histidine	0.20 ± 0.01	0.20 ± 0.02	0.21 ± 0.01
Isoleucine	0.22 ± 0.01	0.22 ± 0.02	0.24 ± 0.00
Leucine	0.84 ± 0.02	0.85 ± 0.03	0.91 ± 0.01
Lysine	0.26 ± 0.01	0.26 ± 0.01	0.28 ± 0.02
Methionine	0.17 ± 0.01	0.17 ± 0.01	0.15 ± 0.00
Phenylalanine	0.34 ± 0.01	0.34 ± 0.03	0.47 ± 0.01
Threonine	0.28 ± 0.01	0.28 ± 0.02	0.29 ± 0.01
Tryptophan	0.06 ± 0.00	0.05 ± 0.00	0.07 ± 0.01
Valine	0.34 ± 0.01	0.34 ± 0.03	0.42 ± 0.02
Dispensable amino acids			
Alanine	0.55 ± 0.02	0.57 ± 0.03	0.56 ± 0.01
Aspartic acid	0.51 ± 0.02	0.49 ± 0.03	0.50 ± 0.01
Cysteine	0.14 ± 0.00	0.14 ± 0.00	0.16 ± 0.00
Glutamic acid	1.37 ± 0.02	1.36 ± 0.04	1.40 ± 0.04
Glycine	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.00
Proline	0.67 ± 0.00	0.70 ± 0.06	0.66 ± 0.01
Tyrosine	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.01
Serine	0.37 ± 0.01	0.37 ± 0.02	0.38 ± 0.02

Abbreviations: ADF, acid detergent fiber; NDF, neutral detergent fiber.
[1]Measured values. Values are means ± SE (n = 3).Corn 0, the fresh corn used in the reference diet; Corn 1, the first test corn sample stored for 3 yr; Corn 2, the second test corn sample stored for 3 yr.

MATERIALS AND METHODS

Corn and Diets

The 2 corn samples were both stored by China Grain Reserves Group Co. Ltd. for 3 yr and were provided by Wellhope Agri-Tech Joint Stock Co. Ltd. The nutrient composition and kernel density of the 2 ingredients are given in Table 1. The composition and nutrient levels of the reference and test diets (fed as mash) are shown in Table 2 and Table 3, respectively. The reference diet was based on corn, soybean meal, corn gluten meal, and soybean oil (Table 2), and the 2 test diets contained 50% of each corn sample and 50% of the reference diet (Table 3).

Calorimetry Chambers

The design of the open-circuit respiratory chamber has been previously described by Liu et al. (2017). Briefly, the respiratory chamber was air-conditioned to maintain the temperature and humidity within 22 to 24°C and 50 to 70% using an air conditioner and a heater installed inside the chamber. Gas was extracted continuously from the respiratory chamber by a vacuum pump. Gas concentrations in each chamber were measured at 21-min intervals by an analyzer. Oxygen was measured with a zirconium oxide sensor (Model 65-4-20; Advanced Micro Instruments, Huntington Beach, CA), whereas CO_2 was measured with a nondispersive infrared sensor (AGM 10; Sensors Europe GmbH, Erkrath, Germany) residing in the analyzer. The analyzer measures a range of 0 to 25% O_2 and 0 to 2.5% of CO_2.

Birds and Experimental Procedures

The experimental procedures for the animal trials were approved by the Animal Ethics Committee of the Jilin Academy of Agricultural Sciences and were performed according to the guidelines for animal experiments set by the National Institute of Animal Health, China.

Hy-Line Brown hens at the age of 35 wk were purchased from the Gong Zhuling local farm in Jilin, China, and were reared following the Hy-Line Brown recommendations (Hy-Line, 2016) in a climate-controlled room. Birds were fed a standard commercial diet and received 16 h of light and 8 h of darkness. A completely randomized block design was used to evaluate 3 diets in 6 respiratory chambers with 3 birds per chamber with 6 repeat runs per diet. Birds were assigned to diets randomly and were 36 to 42 wk of age at the time of the measurement. Birds were adapted to the experimental diets and chamber for 7 D. During the following 3 D, the amount of O_2 consumption and CO_2 production

Table 2. Composition and nutrient levels of the reference diet (as is).

Items	Reference diet
Ingredient (%)	
Corn	61.2
Soybean meal	21.5
Corn gluten meal	3.3
Soybean oil	1.0
Dicalcium phosphate	1.0
Limestone	9.2
Salt	0.3
Premix[1]	2.5
Total	100.0
Measured nutrient levels (%)[2]	
Dry matter	87.1 ± 0.22
Gross energy (kcal/kg)	3,455 ± 6.56
Crude protein	16.0 ± 0.19
Crude fiber	2.4 ± 0.15
Ether extract	3.5 ± 0.21
Calculated nutrient levels (%)	
Calcium	3.8
Avail phosphorus	0.48
Methionine	0.42
Lysine	0.81
Methionine + cysteine	0.73

[1]Provided per kilogram of diet: D,L-methionine, 1.4 g; L-lysine HCL(78.4%), 0.075 g; vitamin A, 10,000 IU; vitamin D3, 3,000 IU; vitamin E (DL-α-tocopheryl acetate), 30 mg; vitamin K3, 1.5 mg; thiamine B1, 2.3 mg; riboflavin B2, 7.8 mg; pyridoxine B6, 5.3 mg; vitamin B12, 0.02 mg; nicotinic acid, 45 mg; pantothenic acid, 12 mg; folic acid, 1.0 mg; biotin, 0.2 mg; Fe, 100 mg; Cu, 20 mg; Zn, 100 mg; Mn, 120 mg; Se, 0.3 mg; I, 1.0 mg.
[2]Values are means ± SE (n = 3).

of laying hens per chamber were determined to calculate HP using the equation reported by Brouwer (1965) without correction for methane and nitrogen in expired gas. The respiration quotient (**RQ**) was determined as the volume of CO_2 produced divided by the volume of O_2 consumed. Measurement was suspended for 2 h each day to replenish feed and collect excreta. The collected excreta were pooled for each chamber over 3 D and stored in a freezer. Initial and final body weight of each laying hen, daily feed intake per chamber, and egg mass and number per chamber were recorded. Feed and water were provided ad libitum at all times.

Analyses of Ingredients, Diets, and Excreta

Samples of corn and diets were dried at 105°C to a constant weight in a forced-air oven to determine the DM content. The 3 D collected excreta per chamber were dried at 105°C to a constant weight for GE and nitrogen analysis. All samples were ground through a mill equipped with a 1-mm screen to ensure a homogeneous mixture. Corn and feed samples were analyzed on an as-fed basis and converted to DM-basis. Gross energy contained in test ingredients, diets, or excreta were measured in a bomb calorimeter (C2000, IKA, Guangzhou, China) using benzoic acid as a standard. Nitrogen was determined with a combustion analyzer (Dumatherm, Gerhardt, Germany) using EDTA as a calibration standard, with CP calculated by multiplying the percentage of nitrogen by a correction factor (6.25). The Soxhlet extraction apparatus with petroleum ether was used for crude fat determination. Total ash was analyzed using a muffle furnace. Crude fiber was determined using the method described in Van Soest et al. (1991). Neutral detergent fiber and acid detergent fiber were analyzed according to the methods of AOAC (2000). The starch content of the corn was analyzed using Method 996.11 of AOAC International (2000). The kernel density of corn was determined using a kernel density measurement instrument for grain (GHCS-1000AP, Shanghai, China) according to the method described in National Standards of the People's Republic of China (GB 1353-2009). The amino acids in the corn samples were determined with a high-speed amino acid analyzer (LA8080, Hitachi, Japan).

Calculation

Egg mass was calculated as a product of hen's day production (%) and the average egg weight (g/bird/D). The feed conversion ratio was calculated as feed intake (g/bird/D) divided by egg mass (g/bird/D). The calculation equations are shown in Table 4. The results for

Table 3. Composition and analyzed nutrient levels of the test diets.

Items	Test diets		Ingredient DM (%)
	Corn 1	Corn 2	
Ingredient (%, as is)			
Reference diet	50.0	50.0	87.1
Corn 1	50.0	-	86.9
Corn 2	-	50.0	86.6
Total	100.0	100.0	
Calculated composition			
Dry matter (%)	87.1	86.8	
Analyzed composition[1]			
Dry matter (%)	86.6 ± 0.42	86.3 ± 0.34	
Crude protein (%)	12.1 ± 0.37	12.2 ± 0.32	
Gross energy (kcal/kg)	3,662 ± 8.97	3,702 ± 14.1	
Ingredients (%, DM)			
Reference diet	50.1	50.1	
Corn 1	49.9	-	
Corn 2	-	49.9	
Total	100.0	100.0	

[1]Values are means ± SE (n = 3).

Table 4. The calculation equations used in the present study.

Numbers	Equations
1	TRN (g/bird/D)[1] = N_i-N_e
2	RN_{egg} (g/bird/D)[2] = 1.936 × Egg mass
3	RN_{body} (g/bird/D)[3] = TRN-RN_{egg}
4	AME_{diet} (kcal/kg DM)[4] = (GE_i-GE_e)/FI
5	$AMEn_{diet}$ (kcal/kg DM)[5] = AME-[8.22 × TRN]/FI
6	AMEI (kcal/brid/D)[6] = AME × FI
7	HP (kcal)[7] = 3.866 × VO_2 (L) + 1.200 × VCO_2 (L)
8	NE_{diet} (kcal/kg DM)[8] = (AMEI-HI)/FI
9	RE (kcal/brid/D)[9] = AMEI-HP
10	$RE_{protein}$ (kcal/bird/D)[10] = TRN × 6.25 × 5.7
11	RE_{fat} (kcal/bird/D)[11] = RE-$RE_{protein}$
12	AME_{corn} (kcal/kg DM)[12] = reference diet AME-[(reference diet AME-test diet AME)/percentage of substitution rate (DM)];
13	$AMEn_{corn}$ (kcal/kg DM)[13] = reference diet AMEn-[(reference diet AMEn-test diet AMEn)/percentage of substitution rate (DM)];
14	NE_{corn} (kcal/kg DM)[14] = reference diet NE-[(reference diet NE-test diet NE)/percentage of substitution rate (DM)].

[1]TRN is the total retained nitrogen, N_i is the nitrogen intake from diet (g/bird/D), and N_e is the nitrogen output from excreta (g/bird/D).
[2]RN_{egg} is the total retained nitrogen in the egg (g/bird/D), 1.936 is N% in the egg (Miranda et al., 2015).
[3]RN_{body} is the total retained nitrogen in body (g/bird/D).
[4]AME_{diet} is the AME value of the diet. GE_i and GE_e are the gross energy intake (kcal/bird/D) from the diet and the gross energy output from excreta (kcal/bird/D), respectively. FI is the feed intake (g/bird/D DM).
[5]$AMEn_{diet}$ is the AMEn value of the diet. 8.22 is the nitrogen correction factor for each gram of nitrogen retained in the body and in eggs (kcal/g; Hill and Anderson, 1958).
[6]AMEI is the AME intake.
[7]HP is the heat production calculated using the following equation, proposed by Brouwer (1965), without correction for methane and nitrogen in expired gas. VO_2 and VCO_2 are volumes of O_2 and CO_2, respectively.
[8]NE_{diet} is the NE value of the diet. HI is heat increment calculated by subtracting FHP from HP. The FHP value of 88 kcal/kg $BW^{0.75}$ per bird per day for laying hens reported by Wu et al. (2016) was used in the calculation. This FHP value corresponds to the asymptotic HP (at zero activity) after a 24-h fasting period.
[9]RE is the retained energy.
[10]$RE_{protein}$ is the retained energy as protein. 6.25 is the protein equivalent of 1 g nitrogen, and 5.7 is the energy equivalent of 1 g protein (kcal/kg/D).
[11]RE_{fat} is the retained energy as fat.
[12]AME_{corn} is the AME value of the corn sample.
[13]$AMEn_{corn}$ is the AMEn value of the corn sample.
[14]NE_{corn} is the NE value of the corn sample.

the AMEI, HP, HI, and retained energy (**RE**) were expressed as kcal/kg $BW^{0.75}$ per bird per day.

Statistical Analysis

The data were analyzed statistically via one-way ANOVA using the GLM procedure in SPSS19.0 (2010, SPSS Inc., Chicago, IL, USA). Statistically significant differences of the 3 dietary treatments were determined using Duncan's multiple-range test. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Birds fed the 2 test diets had a lower ($P < 0.05$) feed intake and average egg weight than the birds fed the reference diet during the 3 D of bioassay (Table 5). This could be the result of the higher energy and lower CP content of the test diets containing 50% corn samples. Keshavarz and Austic (2004) reported that the rate of production, egg weight, egg mass, feed intake, and efficiency of feed conversion of hens in the negative control diet (13% CP) were lower than those of birds fed positive control diet (16% CP). However, dietary treatment did not change the average hen day production, egg mass, and feed conversion ratio ($P > 0.05$) during the bioassay in the present study despite the imbalanced diet. These observations are in agreement with the previous results reported by Barzegar et al. (2019a), 2019b.

Hens fed the test diets had lower ($P < 0.001$) nitrogen intake, nitrogen excreta, and total retained nitrogen compared with those of hens fed the reference diet (Table 5). This difference was expected because the reduced-CP test diets contained less nitrogen than the normal-CP reference diet, and hens fed the 2 test diets consumed lower amounts of feed than the hens fed the reference diet. The nitrogen retained in the body was affected ($P < 0.05$) by different dietary CP contents, while nitrogen retained in the egg was not affected ($P > 0.05$). As shown in Table 5, the total retained nitrogen on average was 1.01 g/bird/D of which 1.00 g/bird/D was exported to the egg with approximately zero-nitrogen deposition in body subsequently. Such nitrogen utilization is consistent with the observation obtained from 16 diets in laying hens in a study by Barzegar et al. (2019a). The 1.01 g/bird/D value of total retained nitrogen in the present study was close to the 0.8 g/bird/D in laying hens reported by Cozannet et al. (2010). With regard to protein intake, it was 18.4 g/brid/D for hens fed the reference diet. This value is slightly greater than Hy-Line (2016) recommendation.

The AMEI, HP, HI, total RE, RE as fat, and RQ of birds were not affected ($P > 0.05$) by different dietary treatments, as shown in Table 6. These results are consistent with previous observations reported by Barzegar et al. (2019a). The HP, HI, and RQ values in the present study were similar to their corresponding values. However, the total RE was greater than the corresponding value in the study by Barzegar et al. (2019a). This may be due to the higher feed intake and AME values of diets in the present study. Birds fed the normal-CP reference diet showed a higher ($P < 0.05$) total energy gain as protein than hens fed the reduced-CP test diets. The AME, AMEn, and NE values of the test diet were higher ($P < 0.05$) than those of the reference diet. The differences between dietary AME and AMEn were 3.7% for the reference diet, 2.2% for the corn 1 test diet, and 2.9% for the corn 2 test diet in the present study. These results are in agreement with the observations reported by Barzegar et al. (2019b). In their study, the AME values of the soybean meal and corn test diets containing 30% test ingredients and 65.7% reference diet were 4 and 3% higher than their AMEn values, respectively.

In the present study, there were no differences in the AME:GE, NE:AME, and NE:AMEn values among the 3 experimental diets. The average dietary efficiencies of

Table 5. Effect of diet composition on performance and N balance in laying hens.[1]

Items	Reference diet	Test diets Corn 1	Test diets Corn 2	SEM	*P*-value
Laying performance					
BW, kg/bird[2]	1.94	1.88	1.90	0.02	0.40
Feed intake, g/bird/D DM	100[a]	81.4[b]	84.4[b]	2.87	0.01
Hen day production, %	88.9	85.2	87.1	3.62	0.93
Average egg weight, g	63.8[a]	56.2[b]	57.3[b]	0.93	<0.001
Egg mass, g/bird/D[3]	57.1	48.0	49.8	2.48	0.30
FCR[4]	1.82	1.80	1.70	0.08	0.85
Nitrogen balance, g/bird/D					
Intake	2.95[a]	1.82[b]	2.06[b]	0.13	<0.001
Excreta	1.60[a]	1.10[b]	1.07[b]	0.07	<0.001
Retained					
Total[5]	1.34[a]	0.71[c]	0.99[b]	0.07	<0.001
Egg[6]	1.11	0.93	0.97	0.05	0.31
Body[7]	0.23[a]	−0.22[b]	0.02[a,b]	0.07	0.02

[a–c]Means within a row lacking a common superscript differ ($P < 0.05$).
Abbreviation: FCR, feed conversion ratio.
[1]Each value represents the mean of 6 replicates (runs) for each treatment (diet) during 3-D respiratory measurements (3 layers per calorimetry chambers).
[2]BW, average body weight.
[3]Egg mass = hen day production × average egg weight (g/bird/D).
[4]FCR (g/g) calculated as feed intake (g) divided by egg mass (g).
[5]Total N retained (g/bird/D) calculated as N intake–N in excreta.
[6]Retained N in egg (g/bird/D).
[7]Retained N in body (g/bird/D) calculated as total N retained - retained N in egg.

GE for AME, AME for NE, and AMEn for NE were 78.4, 75.2, and 77.4%, respectively, in the present study. These results are consistent with the reported average AME:GE value (77%) and NE:AME value (74%) obtained from 16 diets in laying hens (Barzegar et al. 2019a). The AME:GE and NE:AME ratios represent the efficiencies of GE for AME, and AME for NE, respectively. These efficiency values were positively related to EE and starch, and negatively related to CP and crude fiber as shown in broilers (Wu et al., 2018). The efficiencies of AME for NE from EE, starch, and CP were reported to be 84.8, 78.9, and 49.6% in broilers (Wu et al., 2018). The NE:AMEn values of nutrient for broiler chickens were 86.2, 80.6, and 76.0% for lipids, starch, and CP, respectively (Carré et al., 2014).

The GE, AME, AMEn, NE, and energy utilization values of corn are shown in Table 7. The AME values of corn 1 and 2 were 3,485 and 3,675 kcal/kg DM, respectively, without statistical difference observed ($P > 0.05$). Our results were lower than the 3,781 and 3,791 kcal/kg DM, respectively, reported by Celestino et al. (2012) and Barzegar et al. (2019b) in laying

Table 6. Effect of diet composition on energy balance, energy values, and energy utilization in laying hens.

Items	Reference diet	Test diets Corn 1	Test diets Corn 2	SEM	*P*-value
Energy balance, kcal/kg BW$^{0.75}$/bird/D[1]					
AMEI	189	167	177	4.71	0.17
HP	133	130	132	0.64	0.10
HI	45.3	42.0	44.1	0.64	0.10
RE[2]					
Total	55.4	37.1	44.8	4.44	0.25
As protein	29.1[a]	15.8[c]	21.7[b]	1.55	<0.001
As fat	26.4	21.3	23.0	3.55	0.86
RQ[3]	1.006	1.004	1.006	0.002	0.93
Energy values, kcal/kg DM[4]					
AME	3,104[b]	3,294[a]	3,389[a]	36.8	0.001
AMEn	2,993[b]	3,222[a]	3,294[a]	37.5	<0.001
NE	2,357[b]	2,466[a]	2,525[a]	25.8	0.02
Energy utilization, %					
AME:GE	77.8	77.6	79.8	0.60	0.26
NE:AME	76.0	75.0	74.5	0.76	0.74
NE:AMEn	78.8	76.7	76.6	0.78	0.47

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$).
[1]AMEI, apparent metabolizable energy intake; HP, heat production; HI, heat increment.
[2]RE, total retained energy; REegg, retained energy in egg; REbody, retained energy in body.
[3]RQ, respiratory quotient.
[4]GE, gross energy; AME, apparent metabolizable energy; AMEn, AME corrected for zero N retention; NE, net energy.

Table 7. GE, AME, AMEn, NE, and energy utilization values of corn in laying hens.

Items	Corn 1	Corn 2	SEM	*P*-value
$GE_{measured}$, kcal/kg DM[1]	4,511	4,535	-	-
$GE_{calculated}$, kcal/kg DM[2]	4,497	4,501	-	-
AME, kcal/kg DM	3,485	3,675	71.1	0.19
AMEn, kcal/kg DM	3,452	3,596	63.1	0.28
NE, kcal/kg DM	2,575	2,693	59.1	0.34
AME:GE, %	77.5	81.6	1.57	0.20
AMEn:GE, %	76.8	79.9	1.40	0.29
NE:AME, %	74.4	73.3	1.92	0.78
NE:AMEn, %	75.0	74.9	1.89	0.97

[1]$GE_{measured}$, gross energy measured in bomb calorimeter.
[2]$GE_{calculated}$, gross energy calculated by substitution method.

hens, respectively, in the corns. The AME values of 3,785 and 3,775 kcal/kg DM for 2 corn samples determined by the substitution method in broiler breeding cocks in our previous study (Liu et al., 2017) were higher than the values for corn 1 and 2 in the present study.

The AMEn values of corn 1 and 2 derived from the substitution method were 3,452 and 3,596 kcal/kg DM (Table 7), respectively, without showing statistical difference ($P > 0.05$). The AMEn value of 3,722 kcal/kg DM for a corn calculated using the substitution method for laying hens was reported by Barzegar et al. (2019b). Zuber and Rodehutscord (2017) determined the AMEn values of 20 corn samples in laying hens, and the values ranged from 3,752 to 4,087 kcal/kg DM. The GE values of these 20 corn samples were between 4,493 and 4,947 kcal/kg DM. Lessire et al. (2003) reported that the AMEn values of 37 corn samples in cockerels ranged between 3,633 and 4,087 kcal/kg DM. Zhao et al. (2008) found that the AMEn contents of 6 corn samples in adult ducks ranged from 3,633 to 3,776 kcal/kg DM, and their GE values ranged between 4,491 and 4,546 kcal/kg DM. The GE values of corn 1 and corn 2 in the present study were in agreement with these literature values. However, the determined AMEn values of corn 1 and 2 were lower than these values.

The NE values of corn 1 and 2 were 2,575 and 2,693 kcal/kg DM (Table 7), respectively, without showing statistical difference ($P > 0.05$). Barzegar et al. (2019a) estimated the AME and NE values of corn by multiple linear regression equation, and the values were 4,024 and 3,008 kcal/kg DM, respectively. Their AME and NE values were higher than the values of corn 1 and 2 in the present study. We calculated the NE values of the 2 corn samples using the regression equation 15 ($NE = 0.781 \times AME - 11 \times CP + 16.4 \times EE$) reported by Barzegar et al. (2019a). The calculated NE values (2,682 and 2,829 kcal/kg DM) are close to the measured NE values (2,575 and 2,693 kcal/kg DM) for corn 1 and corn 2, respectively. The NE values of the 2 corn samples in the present study are lower than 2,982 and 3,006 kcal/kg DM previously reported by Liu et al. (2017) for 2 corn samples determined using the substitution method in broiler breeding cocks.

As described previously, the AME, AMEn, and NE values of the 2 corn samples were lower than the literature values for fresh corn, while the corresponding GE values were in agreement with these reported values. The decreased available energy values of the corn samples may be caused by the storage of the grains. The content of starch, CP, and amino acids in corn decreases with storage time (Yin et al., 2017). The corn storage might cause starch retrogradation and lead to resistant starch formation resulting in a decreased nutritional value of starch (Garcíarosas et al., 2010). Yin et al. (2017) reported that the performance of broilers decreased using corn stored for 4 yr to formulate feed. The differences in energy values could also be due to differences in corn hybrids and growing conditions at the specific growing location (field).

The efficiencies of GE for AME were 77.5% for corn 1 and 81.6% for corn 2 (Table 7). Barzegar et al. (2019b) reported that the efficiency of GE for AME was 85.4% for corn in laying hens. In another study by Barzegar et al. (2019a), the AME:GE value of 81.8% for corn in laying hens was higher than that value of corn 1 (77.5%) and was close to 81.6% for corn 2 in the present study. The efficiencies of GE for AMEn were 76.8% for corn 1 and 79.9% for corn 2, respectively (Table 7). The AMEn:GE values for corn 1 and corn 2 determined in the present study were approximately 7 and 4% lower than these values for corn (83.6 and 83.9%) observed by Barzegar et al. (2019b) in laying hens, respectively. The NE:AME ratios were 74.4 and 73.3% for corn 1 and corn 2 (Table 7), respectively. The efficiencies of NE for AMEn were 75.0 and 74.9% for corn 1 and corn 2 (Table 7), respectively. Barzegar et al. (2019a) reported the NE:AME ratio of corn was 74.7%, and the NE:AMEn ratio was 77.7% in laying hens. Wu et al. (2018) reported that the NE:AMEn value of corn was 80.6% in broilers. The NE:AME values of starch were 78% in laying hens reported by Barzegar et al. (2019a), 79% in broilers observed by Wu et al. (2018), and 78% in broilers reported by Carré et al. (2014).

In conclusion, the AME, AMEn, and NE values of the 2 corn samples both stored for 3 yr were determined by the reference diet substitution method in laying hens. The AME values of the 2 corn samples were 3,485 and 3,675 kcal/kg DM, respectively. The corresponding AMEn values were 3,452 and 3,596 kcal/kg DM, and NE values were 2,575 and 2,693 kcal/kg DM, respectively. The AME, AMEn, and NE values of the 2 corn samples both stored for 3 yr were lower than the literature values for fresh corn. The outcomes of the present study could provide a reference for the formulation of laying hen feed using stored corn by ME and NE systems.

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