Original Research Article

Comparative study of apparent metabolizable energy and net energy values of dephenolized cottonseed proteins for laying hens

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ABSTRACT

Optimizing the energy utilization of nutrients and ensuring maximum benefits are continuous goals for livestock producers. The net energy (NE) value of feed reflects its nutritional value in the precision feeding system. An experiment was conducted to determine the apparent metabolizable energy (AME) and NE values of 3 types of dephenolized cottonseed protein (DCP) for Hy Line Brown hens aged 42 to 45 weeks using the reference diet substitution method. A reference diet based on corn soybean meal was used to meet the nutritional needs of Hy Line Brown laying hens. To render the crude protein and energy values of the 3 test diets similar, 10.5%, 12%, and 16% of the gross energy yielding ingredients from the reference diet were replaced with DCP 1, DCP 2, and DCP 3, respectively. The birds were fed 4 diets during a 7-d adaptation period. After the dietary adaptation period, 2 birds per replicate from each treatment group were placed in an individual open circuit respiratory calorimetry chamber for a 3-d experimental period. Daily O_2 consumption and CO_2 production were recorded, and excreta samples were collected. The AME values of DCP 1, DCP 2, and DCP 3 were 3,049.05, 2,820.13, and 2,982.31 kcal/kg of dry matter (DM), respectively. The NE values of DCP 1, DCP 2, DCP 3 were 1,475.77, 1,910.31, and 1,905.37 kcal/kg of DM, respectively, and the NE:AME ratios were 48.40%, 67.74%, and 63.89%, respectively. Our data show that the AME value of DCP does not reflect the nutritional value of the feed. The NE value of DCP with a high ME value was not necessarily high.

1. Introduction

Poultry breeding has resulted in considerable differences in endocrine, nutrient digestion, and energy utilization efficiency between broiler and layer type chickens (Koenen et al., 2002; Buzala et al., 2015; Adeola et al., 2018). Adedokun et al. (2015) observed that ileal digestibility of crude protein and standardized ileal amino acid digestibility of corn distillers dried grain with solubles was higher ($P < 0.05$) in 21-day-old broilers than in laying hens. Pishnamazi et al. (2005) observed that the apparent metabolizable energy (AME) corrected to zero nitrogen retention (AMEn) in broiler chickens for corn and wheat bran was lower than that in White Leghorn birds. Therefore, an accurate evaluation of feed energy is crucial for the rational selection of feed raw materials, optimizing feed formulas, and reducing feed costs. However, research on the energy of feed materials for laying hens is limited.

The net energy (NE) and the feeding values of feed raw materials with the same metabolizable energy (ME) value may differ. NE is calculated by subtracting the heat increment (HI) component from the value of ME (Noblet and van Milgen, 2004; Ning et al., 2014). According to Barekatain et al. (2014), in broilers fed 2 diets with the same ME and crude protein, birds fed diets containing DDGS had lower NE, higher heat production (HP), and lower weight during starter and grower phases. Therefore, NE is more accurate than ME

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in reflecting the nutritional value of feed (Carré et al., 2014; Swick et al., 2014).

In China, cotton yield is high, and the price of cottonseed is stable. The by-product of cottonseed processing, dephenolized cottonseed protein (DCP), is a commonly used protein feedstuff (Ma et al., 2018). Previous research has shown that the de gossypol procedure helps improve the palatability and digestibility of cottonseed by eliminating free gossypol and other harmful substances, thus making it easier for animals to digest and utilize. However, there are few reports on the NE value of DCP in laying hens, limiting its application in laying hen feed. Therefore, this study aimed to measure and compare the AME and NE of 3 types of DCP.

2. Materials and methods

The study protocol was approved by the Animal Ethics Committee of China Agricultural University (AW02501202-2-1). Furthermore, the study was conducted in accordance with the guidelines of the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

2.1. DCP and diets

Dephenolized cottonseed protein was obtained from the Xinjiang Province, China. The reference diet was based on corn soybean meal and met the nutrient requirements of laying hens. Three types of DCPs (DCP 1, DCP 2, and DCP 3) with crude protein content of 66.79%, 62.99%, and 51.53% were used in the test diets to replace 10.5%, 12%, and 16% of the gross energy (GE) yielding ingredients from the reference diet, respectively. The substitution ratios were used to keep the crude protein content consistent across the 3 test diets. To maintain consistency, vitamins, minerals, and other non-energy ingredients were added to the reference and test diets. The birds had ad libitum access to water and feed. Before preparing the feed, the moisture content of each ingredient was determined to calculate its contribution to each diet on a dry matter (DM) basis. The ingredients and chemical composition of the diets used in the experiment are presented in Table 1.

2.2. Equipment

An open circuit respiratory calorimeter chamber with a volume of approximately 0.54 m^3 was used to measure poultry O_2 consumption and CO_2 production. Oxygen content was measured using a zirconium oxide sensor (Model 65-4-20; Advanced Micro Instruments Inc., Huntington Beach, CA, USA), and CO_2 content was measured using a non-dispersive infrared sensor (AGM 10; Sensors Europe GmbH, Erkrath, Germany). In addition, a cage measuring 70 cm × 55 cm × 70 cm, equipped with feeders and nipple drinkers, was placed inside the calorimeter.

2.3. Animals and experimental design

Hy Line Brown pullets were purchased from a commercial hatchery. Layer chickens were raised according to the management handbook (Hy-Line, 2018). The experiment was initiated when laying hens reached 42 wk of age. Birds weighing 1.9 ± 0.1 kg and laying at 91% to 93% hen day production (HDP) were selected. Since the number of chambers was not enough to complete the experiment in one run, the experiment was conducted thrice, each time using different laying hens.

A completely randomized design was used for each experiment to evaluate 4 different diets in 8 chambers (2 chambers per diet) with 2 birds. The number of birds in each chamber was determined

Table 1
Ingredients and chemical composition of the diets used in the study (%, as is basis).

Item	Reference diet	Test diets		
		DCP 1	DCP 2	DCP 3
Ingredients				
Corn	61.20	54.77	53.86	51.41
Soybean meal	21.50	19.24	18.92	18.06
Corn gluten meal	3.30	2.95	2.90	2.77
Soybean oil	1.00	0.90	0.88	0.84
Dephenolized cottonseed protein		9.13	10.44	13.92
Limestone	8.00	8.00	8.00	8.00
Compound premix (5.0%)[1]	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00
Nutrient levels (calculated)				
Crude protein	17.00	21.05	21.16	20.96
Moisture	12.33	12.15	12.06	12.00
Crude fat	3.73	3.47	3.24	2.99
Crude fiber	2.40	2.72	2.86	3.86
NDF	9.66	11.60	12.66	15.36
Starch	40.98	38.45	36.28	34.39
Ca	3.80	3.80	3.80	3.80
Total P	0.42	0.42	0.42	0.42
Methionine	0.42	0.57	0.70	0.84
Lysine	0.81	1.03	1.19	1.37
Threonine	0.66	0.80	0.92	1.04

DCP 1 = dephenolized cottonseed protein with 66.79% crude protein content (dry matter basis); DCP 2 = dephenolized cottonseed protein with 62.99% crude protein content (dry matter basis); DCP 3 = dephenolized cottonseed protein with 51.53% crude protein content (dry matter basis); NDF = neutral detergent fiber.
[1] Provided per kilogram of diet: D,L-methionine, 2.8 g; L-lysine HCl (78.4%), 0.15 g; vitamin A, 20,000 IU; vitamin D$_3$, 6,000 IU; vitamin E (DL-α-tocopheryl acetate), 60 mg; vitamin K$_3$, 3 mg; thiamine, 4.6 mg; riboflavin, 15 mg; pyridoxine, 10.6 mg; vitamin B$_{12}$, 0.04 mg; nicotinic acid, 90 mg; pantothenic acid, 24 mg; folic acid, 2.0 mg; biotin, 0.4 mg; Fe, 200 mg; Cu, 40 mg; Zn, 200 mg; Mn, 240 mg; Se, 0.6 mg.

according to bird size, chamber capacity, and CO_2 concentration in the chamber.

The experiment included adaptation and experimental periods. During the adaptation and experimental periods, chamber temperature was maintained at 20 to 22 °C, and air humidity was maintained at 60% to 70%. The lights were switched on at 04:00 and off at 20:00, thus completing a 16-h light and 8-h dark cycle. At the beginning of the adaptation period, birds were fed test diets and, on the 4th day, moved from the coops to the chambers, with chamber lids open and air pumps running in a climate controlled room. During the experimental period, feed intake (FI) was measured, total excreta were collected, eggs were collected daily and used to record HDP and O_2 consumption and CO_2 production were recorded. Feed spillage from the under cage collection tray was measured and subtracted from the FI. In addition, feathers were removed from the collected excreta (Ning et al., 2014; Barzegar et al., 2019b).

2.4. Analyses of ingredients, diets, and excreta

During the experimental period, a feed sample for each diet was collected, and its DM content was measured and subsequently used for chemical analyses. Daily excreta were stored in a refrigerator at 4 °C. At the end of each period, excreta from each respiratory chamber was mixed and dried in an oven at 65 °C for 72 h. After 72 h, excreta were left at room temperature (25 °C), crushed, and then analyzed for crude protein, crude fat, crude fiber, ash, neutral detergent fiber, and acid detergent fiber (AOAC, 2016). The gross energy (GE) contained in the ingredients of the test diets and excreta was measured using a bomb calorimeter (C2000; IKA, Guangzhou, China) with benzoic acid as a standard. The chemical characteristics of DCP are shown in Table 2.

Table 2
Chemical characteristics of dephenolized cottonseed protein (DCP) (DM basis).

Item	DCP 1	DCP 2	DCP 3
Crude fat, %	0.96	1.40	1.65
Crude protein, %	66.79	62.99	51.53
Ash, %	10.88	11.02	9.82
Crude fiber, %	6.53	4.65	11.37
Nitrogen free extract, %	14.84	19.94	25.63
NDF, %	34.53	24.49	36.23
ADF, %	6.89	5.99	14.95
Gross energy, kcal/kg DM	4,723.69	4,776.46	4,871.72

DM = dry matter; DCP 1 = dephenolized cottonseed protein with 66.79% crude protein content (dry matter basis); DCP 2 = dephenolized cottonseed protein with 62.99% crude protein content (dry matter basis); DCP 3 = dephenolized cottonseed protein with 51.53% crude protein content (dry matter basis); NDF = neutral detergent fiber; ADF = acid detergent fiber.

2.5. Calculations

The measured poultry O_2 consumption and CO_2 production were used to calculate HP calculated as follows: HP (kcal) = $3.866 \times VO_2$ (L) + $1.200 \times VCO_2$ (L) (Brouwer, 1965), where VO_2 is the volume of O_2 consumed, and VCO_2 is the volume of CO_2 exhaled.

The metabolizable energy intake (MEI), retained energy (RE), nitrogen retained (NR), heat increment (HI), and respiratory quotient (RQ) of the experimental diets were determined using the following equations:

$$MEI \ (kcal/d) = (FI \times GEd) - (E \times GEe)$$

$$RE \ (kcal/d) = MEI - THP$$

$$NR \ (g/d) = Ni - Ne$$

$$HI \ (kcal/d) = THP - FHP$$

$$RQ = VCO2 / VO2$$

where FI is the feed intake (kg of DM), E is the excreta output (kg of DM), GEd is the gross energy of the diet (kcal/kg of DM); GEe is the gross energy of the excreta (kcal/kg of DM), THP is the total heat production (kcal/d), Ni is the nitrogen intake from the diet (g/d), and Ne is the nitrogen excretion through the excreta (g/d). To correct for the effect of body weight on energy metabolism and respiration data between animals, the data were converted to $BW^{0.75}$. The present study used a fasting heat production (FHP) value of 370 kJ/$BW^{0.75}$ per d per bird for layer hens (Wu et al., 2016). The formulae for calculating the AME, AMEn, and NE of the experimental diets and DCP used in the study have been previously described (Liu et al., 2022).

2.6. Statistical analyses

Production performance, nitrogen balance, and energy metabolism data obtained during the 3 d experimental period were analyzed using a two-factor ANOVA with "diet" and "week age" as fixed factors in SPSS (SPSS Inc., Chicago, IL, USA). Then used the main effect of "diet" for the results. Differences between treatment groups were examined using the LSD and were considered statistically significant at $P < 0.05$.

3. Results

The chemical characteristics of DCP are shown in Table 2. The neutral detergent fiber and acid detergent fiber content were found to be numerically greater in DCP 3 than in the other 2 DCP types. The crude protein content was found to be higher in DCP 1 than in the other 2 DCP types.

The AME, AMEn, and NE values and NE:AME ratios of DCP 1, DCP 2, and DCP 3 are shown in Table 3. The AME values of DCP were 3,049.05, 2,820.13, and 2,982.31 kcal/kg of DM (for DCP 1, DCP 2, and DCP 3), respectively. The AMEn values of DCP were 2,875, 2,534.77, and 2,721.21 kcal/kg of DM, respectively. The NE values were 1,475.77, 1,910.31, and 1,905.37 kcal/kg DM, respectively. The NE:AME ratios of DCP were 48.40%, 67.74%, and 63.89%, respectively.

As shown in Table 4, the 4 diets had no substantial effect on FI, HDP, VO_2, VCO_2, and RQ. However, the DCP 2 group significantly increased its nitrogen intake ($P < 0.01$), and increased excretion ($P < 0.05$) and retention ($P < 0.05$), while the DCP 3 group significantly increased its nitrogen intake ($P < 0.01$) and retention ($P < 0.05$). No significant effect was observed in the DCP 1 group ($P > 0.05$).

Compared with the reference diet, DCP 2 and DCP 3 increased the retained energy (RE) as protein ($P < 0.05$) but had no effect on GE, AME, and NE intake, total heat production (THP), and RE. In addition, there were no significant differences in the AME:GE and NE:AME ratios in the energy utilization of the experimental diets in laying hens ($P > 0.05$; Table 5).

4. Discussion

The analyzed nutrient contents and GE in the DCP treatments were within the range of data reported by Gerasimidis et al. (2009) and He et al. (2015). Meals with DCP have higher crude protein content than that of ordinary cottonseed meals (He et al., 2015; Świątkiewicz et al., 2016). To our knowledge, this is the first report on the NE value of high protein DCP in Hy Line Brown hens. The results show differences in ME, NE, and energy efficiency for different DCPs. DCP 1 had the highest crude protein content, AME, AMEn, and AME:GE value; however, the NE value and NE:AME for DCP 1 were the lowest among the 3 DCPs. In laying hens, the NE:AME efficiency ratios were 49% and 108% for CP and EE, respectively (Barzegar et al., 2019a). The large difference in NE:AME among the 3 DCPs may be due to 2 factors: on the one hand, compared with fat, the metabolism and turnover of protein in laying hens require more energy to fuel metabolic pathways, at the same time, protein catabolism leads to nitrogenous wastes that

Table 3
Energy value and conversion efficiency of dephenolized cottonseed protein (DCP) observed in the study (DM basis).

Item	DCP 1	DCP 2	DCP 3
AME, kcal/kg DM	3,049.05	2,820.13	2,982.31
AMEn, kcal/kg DM	2,875.58	2,534.77	2,721.21
NE, kcal/kg DM	1,475.77	1,910.31	1,905.37
AME/GE, %	64.55	59.04	61.22
NE/AME, %	48.40	67.74	63.89

DM = dry matter; DCP 1 = dephenolized cottonseed protein with 66.79% crude protein content (dry matter basis); DCP 2 = dephenolized cottonseed protein with 62.99% crude protein content (dry matter basis); DCP 3 = dephenolized cottonseed protein with 51.53% crude protein content (dry matter basis); AME = apparent metabolizable energy; AMEn = AME corrected zero nitrogen retention; NE = net energy; GE = gross energy.

Table 4
Effect of diet composition on performance and nitrogen balance in laying hens.[1]

Item	Reference diet	Test diets			SEM	*P*-value
		DCP 1	DCP 2	DCP 3		
Performance						
Average body weight, g	1,927.15	1,801.42	1,930.92	1,873.65	22.79	0.056
Body weight gain, g	−88.40	−76.83	−71.16	−81.10	6.91	0.823
Feed intake, g of DM/d	82.73	81.06	91.75	86.41	1.67	0.139
HDP, %	88.33	83.33	91.67	90.00	2.49	0.830
Respiratory						
VO_2, L/d per kg $BW^{0.75}$	25.94	26.83	26.88	26.81	0.45	0.650
VCO_2, L/d per kg $BW^{0.75}$	25.88	26.59	26.59	26.38	0.46	0.450
Respiratory quotient, L/L	1.00	0.99	0.99	0.98	0.01	0.939
Nitrogen intake, g/d	3.02[c]	3.20[b,c]	3.85[a]	3.57[a,b]	0.09	0.001
Nitrogen excretion, g/d	2.09[b]	2.20[b]	2.59[a]	2.36[a,b]	0.06	0.020
Nitrogen retained, g/d	0.94c	1.00[b,c]	1.25[a]	1.21[a,b]	0.04	0.012

DCP 1 = dephenolized cottonseed protein with 66.79% crude protein content (dry matter basis); DCP 2 = dephenolized cottonseed protein with 62.99% crude protein content (dry matter basis); DCP 3 = dephenolized cottonseed protein with 51.53% crude protein content (dry matter basis); SEM = standard error of means ($n = 6$); DM = dry matter; HDP = hen day production.
[a to c] Means withour a common superscript are significantly different from each other at $P < 0.05$.
[1] Values are means of 6 replicate pens with 2 birds/pen at placement.

Table 5
Effects of different diets on energy metabolism in laying hens.[1]

Item	Reference diet	Test diets			SEM	*P*-value
		DCP 1	DCP 2	DCP 3		
Energy balance, kcal/kg $BW^{0.75}$ per d						
THP	131.28	135.58	135.74	135.25	2.22	0.591
HI	42.90	47.21	47.37	46.88	2.22	0.591
RE	22.77	21.29	31.66	24.85	2.71	0.640
RE as protein	20.46[b]	22.93[a,b]	27.37[a]	26.79[a]	1.02	0.010
RE as fat	2.31	−1.64	4.29	−1.94	2.54	0.843
GEI	212.54	220.30	236.86	228.27	4.07	0.154
AMEI	154.05	156.87	167.41	160.10	2.99	0.395
NEI	111.14	109.66	120.04	113.22	2.71	0.640
Available energy, kcal/kg DM						
AME	3,049.28	3,003.89	2,973.43	2,970.80	14.08	0.169
AMEn	2,955.58[a]	2,904.22[a,b]	2,861.27[b]	2,855.98[b]	13.17	0.025
NE	2,200.29	2,100.65	2,132.52	2,109.42	38.17	0.658
Efficiency, %						
AME/GE	72.47	71.12	70.63	70.36	0.35	0.096
NE/AME	72.15	70.00	71.71	71.31	1.28	0.843

DCP 1 = dephenolized cottonseed protein with 66.79% crude protein content (dry matter basis); DCP 2 = dephenolized cottonseed protein with 62.99% crude protein content (dry matter basis); DCP 3 = dephenolized cottonseed protein with 51.53% crude protein content (dry matter basis); SEM = standard error of means ($n = 6$); THP = total heat production; HI = heat increment; RE = retained energy; GEI = gross energy intake; AMEI = apparent metabolizable energy intake; NEI = net energy intake; DM = dry matter; AME = apparent metabolizable energy; AMEn = AME corrected zero nitrogen retention; NE = net energy; GE = gross energy.
[a, b] Means without a common superscript are significantly different from each other at $P < 0.05$.
[1] Values are means of 6 replicate pens with 2 birds/pen at placement.

require energy to be excreted (Musharaf and Latshaw, 1999). On the other hand, the fiber content of raw materials also has a negative impact on NE (Barzegar et al., 2020b). DCP 1 has a high content of crude protein and NDF, which may be the reason for its low NE. In addition, the FI and HDP are much lower, reflecting that palatability and energy/protein is low for DCP 1. Although the gossypol content of DCP 1 is low, less than 0.04%, it is uncertain which other antigenic factors, such as tannin or phytic acid, are present. Furthermore, poor palatability for laying hens due to its finer powder form than that of the other 2 DCPs could affect NE. The basic assumption for using the substitution method to determine the energy values of the ingredients is that there is no interaction between the test ingredients and reference diet (Barzegar et al., 2020b). However, previous studies have shown that the nutritional level of the reference diet and substitution ratio can affect the results to a certain extent (Mateos et al., 2019; Wu et al., 2020). Liu et al. (2021) reported that the energy values of high fiber ingredients determined by the substitution method with lower substitution ratios were inconsistent with the values estimated by the regression method. The difference in substitution ratio of the 3 DCPs in the test diets may also be one of the reasons for the difference in NE; this needs to be verified by subsequent experiments.

In the present study, results on the effects of diet composition on performance and nitrogen balance in laying hens indicated that layer hen diets containing no more than 16% DCP had no negative effect on its performance. This shows that the dephenolization of cottonseed protein greatly reduced its anti-nutritional effect. Wang et al. (2015) reported that half replacement of cottonseed protein combined with soybean meal could maintain egg quality similar to that of a soybean meal diet. Furthermore, He et al. (2015) reported that 50% soybean meal, when replaced with low gossypol cottonseed meal with optimum feed conversion ratio, had no adverse effects on egg production, quality, and health of layer hens.

The results of the present study show that the respiration and HP of metabolites of the test diet group were higher than those in the reference diet group. The principal reason is that the crude protein content in the test diet was higher than that in the reference diet. Previous studies have shown that the respiratory

capacity, THP, and HI of poultry increased with an increase in dietary crude protein content (Wu et al., 2019; Barzegar et al., 2020a; Choi et al., 2021). In the present study, RE as protein in the test diet group was significantly higher than that in the reference diet group, indicating that the form of energy provided by the feed could affect the form of energy deposited in the body. Our results suggest that after an in depth study of feed NE and animal energy distribution, we can change the nutritional composition of eggs and chicken by adjusting the form and quantity of energy supply to the animal.

5. Conclusion

High protein DCP has a high nutritional value and has no negative effects on the production of laying hens. Therefore, it can be used to formulate laying hen feed after its effective energy and nutrient content evaluation. Furthermore, the AME value of DCP does not reflect the nutritional value of the feed, and the NE value of DCP with a high ME value is not necessarily high.

Author contributions

Yongfa Liu: Conceptualization, Methodology, Writing - Original Draft & Editing; **Yi Wei**: Data curation, Writing − Editing; **Qiuyu Jiang**: Data curation, Writing − Editing; **Peng Li**: Resources, Writing − Editing; **Zhibin Ban**: Resources, Supervision, Writing - Review & Editing, Project administration, Funding acquisition; **Zengpeng Lv**: Formal analysis, Writing - Review & Editing, Project administration; **Yuming Guo**: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

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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