

Postprint: Determination of Net Energy of Cottonseed Meal Using British Strain Pekin Ducks

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Abstract

This study aimed to evaluate the net energy (NE) of cottonseed meal in British Beijing ducks and establish its prediction equation using the apparent metabolizable energy (AME) and routine components of cottonseed meal. According to the factorial method, the NE of cottonseed meal substitution diets was partitioned into net energy for maintenance (NEm) and net energy for production (NEp). NEm was determined using the regression method, NEp was determined using the comparative slaughter method, and the NE of cottonseed meal was then calculated by difference. Four hundred 7-day-old British Beijing ducks were selected for the animal experiment. First, 20 ducks were selected and killed by cervical dislocation at the beginning of the experiment to determine their body energy, which served as the common initial energy control for determining net energy for maintenance (NEm) and net energy for production (NEp). Another 50 ducks were selected for NEm determination; NEm was estimated using fasting heat production (FHP). There were 5 groups (5 replicates per group, 2 ducks per replicate), including an ad libitum feeding group and feed restriction groups of 15%, 25%, 35%, and 45%, all fed basal diet 1. The remaining 330 ducks were used for NEp determination, with 33 groups (5 replicates per group, 2 ducks per replicate) fed either basal diet 2 or experimental diets in which different cottonseed meals substituted 15% of basal diet 2. At 14 days of age, all experimental ducks were killed by cervical dislocation to determine their body energy. Finally, correlation analysis and multiple stepwise linear regression analysis were performed between the measured NE of cottonseed meal and its AME and routine components to establish a prediction equation for the NE of cottonseed meal. The results showed that the FHP of British Beijing ducks was 580.7 kJ/(kg W^{0.75} · d). The NEm and NEp provided by cottonseed meal substitution diets to ducks were $(2.97 \pm 0.15) \text{ MJ/kg}$ and $(5.22 \pm 0.12) \text{ MJ/kg}$, respectively. The NE provided by cottonseed meal substitution diets to ducks was 5.04~7.30 MJ/kg, with a distribution range of 5.04~7.30 MJ/kg, showing considerable

variation. The NE of cottonseed meal for British Beijing ducks can be accurately predicted using AME combined with routine components.

Full Text

Study on Evaluating the Net Energy of Cottonseed Meal for Cherry Valley Ducks

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Abstract

This study aimed to evaluate the net energy (NE) of cottonseed meal in Cherry Valley ducks and establish prediction equations for NE using apparent metabolizable energy (AME) and conventional composition. The factorial method was employed to partition the NE of cottonseed meal-substituted diets into net energy for maintenance (NEm) and net energy for production (NEp). NEm was measured using the regression method, while NEp was determined via comparative slaughter methodology, and the NE of cottonseed meal was subsequently calculated. A total of 400 seven-day-old Cherry Valley ducks were used in the animal experiment. Initially, 20 ducks were sacrificed by cervical dislocation to determine initial body energy, serving as a common baseline for both NEm and NEp measurements. Fifty ducks were allocated to NEm determination across five groups (five replicates of two ducks each): an ad libitum feeding group and groups with feed restriction at 15%, 25%, 35%, and 45% levels, all receiving basal diet 1. The remaining 330 ducks were used for NEp determination, distributed across 33 groups (five replicates of two ducks each) fed either basal diet 2 or experimental diets where different cottonseed meals replaced 15% of basal diet 2. All experimental ducks were sacrificed by cervical dislocation at 14 days of age to determine body energy. Finally, correlation analysis and multiple stepwise linear regression were performed between the measured NE values of cottonseed meal and its AME and conventional composition to establish prediction equations.

The results showed that the fasting heat production (FHP) of Cherry Valley ducks was $580.7 \text{ kJ}/(\text{kg W}^{0.75} \cdot \text{d})$. The NEm and NEp provided by cottonseed meal-substituted diets were $(2.97 \pm 0.15) \text{ MJ/kg}$ and $(5.22 \pm 0.12) \text{ MJ/kg}$, respectively, yielding a total NE of $(8.19 \pm 0.17) \text{ MJ/kg}$. The NE conversion efficiency of cottonseed meal was $(60.97 \pm 2.04)\%$. The optimal prediction equation established using AME and conventional composition was:

$$NE = 3.276 + 0.241AME + 0.044CP - 0.081ADF \quad (R^2 = 0.954, RSD = 0.13 \text{ MJ/kg}, P < 0.01)$$

where CP is crude protein and ADF is acid detergent fiber. In conclusion, the NE of cottonseed meal for Cherry Valley ducks is (6.12 ± 0.62) MJ/kg, ranging from 5.04 to 7.30 MJ/kg with substantial variation. The NE can be accurately predicted using AME combined with conventional composition.

Keywords: cottonseed meal; net energy; ducks; conventional composition; prediction

Introduction

Currently, the metabolizable energy (ME) system is commonly used for poultry energy metabolism evaluation. However, this system tends to overestimate the effective energy of protein and fiber in feedstuffs [1]. Net energy (NE), defined as ME minus heat increment, can eliminate the effects of specific feed components on effective energy values and thus provides a more accurate assessment than the ME system. Evaluation of feed NE represents a frontier and hotspot in feed science research. Previous studies have determined the NE of cottonseed meal in yellow-feathered broilers and Avian broilers [2-3], and have found that NE correlates with apparent metabolizable energy (AME) and conventional composition. For example, Noblet et al. [1] established NE prediction equations using AME and conventional composition that are widely used for rapid evaluation of NE in growing pig feed ingredients and compound diets [4-5]. However, no studies have reported the NE of cottonseed meal in meat ducks, which exhibit distinctly different energy utilization efficiency compared to broilers, and the relationship between its NE and conventional composition remains unclear. Therefore, this study selected Cherry Valley ducks as an animal model to determine the AME and NE of cottonseed meal from different sources and to investigate the correlation and regression relationships between NE and AME as well as conventional composition, thereby providing a basis for rapid and accurate NE prediction and promoting efficient utilization of cottonseed meal.

Materials and Methods

1.1 Cottonseed Meal Collection

Thirty-two cottonseed meal samples were collected from different provinces including Xinjiang, Shandong, Henan, Hebei, Jiangsu, Jiangxi, and Ningxia, based on cottonseed meal production regions and feed standards. The dry matter (DM), crude protein (CP), crude ash (Ash), ether extract (EE), crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF) contents were determined according to GB/T 6435-2014, GB/T 6432-1994, GB/T 6438-2007, GB/T 6433-2006, GB/T 6434-2006, NY/T 1459-2007, and GB/T 20806-2006, respectively.

1.2 Experimental Design

Cherry Valley ducks were purchased from the Institute of Animal Genetics and Breeding at Sichuan Agricultural University. Newly hatched ducks from the same batch were immediately transferred to the metabolism room for conventional rearing management. At 7 days of age, 400 healthy ducks with an average fasting body weight of (130.0 ± 8.5) g were selected for a 7-day metabolism trial. At the trial's onset, 20 ducks were sacrificed by cervical dislocation and stored at -20°C to determine initial body energy, serving as a common baseline for both NEm and NEp measurements. Another 50 ducks were allocated to NEm determination across five groups (five replicates of two ducks each): an ad libitum group and groups with 15%, 25%, 35%, and 45% feed restriction, all fed basal diet 1. The remaining 330 ducks were used for NEp determination, distributed across 33 groups (five replicates of two ducks each) fed either basal diet 2 or experimental diets where different cottonseed meals replaced 15% of basal diet 2. At 12.5 days of age, all ducks were fasted for 36 hours and sacrificed by cervical dislocation at 14 days of age, then stored at -20°C .

1.3 Basal Diets

Basal diet 1 was formulated according to NY/T 2122-2012 (Feeding Standard of Meat Ducks) for NEm determination. To meet protein level requirements after cottonseed meal substitution, the protein level of basal diet 2 used for NEp determination was reduced by 4 percentage points. The composition and nutrient levels of basal diets are presented in Table 1. All experimental diets were pelleted.

Table 1 Composition and nutrient levels of basal diets (air-dry basis) %

Items	Basal diet 1	Basal diet 2
Ingredients		
Corn		
Soybean meal (solvent extracted)		
Cottonseed meal (solvent extracted)		
Soybean oil		
L-Lys · HCl		
DL-Met		
Limestone		
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$		
NaCl		
Choline chloride (50%)		
Premix ¹		
Total		
Nutrient levels²		
ME (MJ/kg)		
CP		

Items	Basal diet 1	Basal diet 2
Ca		
TP		
Lys		
Met		
Met+Cys		
Thr		
Trp		

¹The premix provided per kg of diet: VA 4,000 IU, VB₁ 2 mg, VB₂ 10 mg, VB₆ 4 mg, VB₁₂ 0.02 mg, VK 2 mg, folic acid 1 mg, calcium pantothenate 20 mg, nicotinic acid 50 mg, Cu (as copper sulfate) 4 mg, Fe (as ferrous sulfate) 60 mg, Mn (as manganese sulfate) 30 mg, Zn (as zinc sulfate) 35 mg, I (as potassium iodide) 0.4 mg, Se (as sodium selenite) 0.25 mg.

²Nutrient levels were calculated values.

1.4 Management

The trial was conducted at the Waterfowl Metabolism Room of the Animal Nutrition Institute Farm, Sichuan Agricultural University. Ducks were raised on wire floors and fed twice daily at 08:00 and 20:00. During the first week, room temperature was maintained at $(30 \pm 2)^{\circ}\text{C}$ using heat lamps; during the formal experimental period, temperature was maintained at $(20 \pm 2)^{\circ}\text{C}$. Ducks had ad libitum access to water via nipple drinkers, 24-hour lighting, and relative humidity of 60%-65%. All groups for NEm determination were fed basal diet 1; the ad libitum group's feed allowance was adjusted based on previous intake to ensure 10% leftover feed, while restricted groups received 85%, 75%, 65%, and 55% of ad libitum intake for the 15%, 25%, 35%, and 45% restriction levels, respectively.

1.5 Measurements

Apparent metabolizable energy was determined using the partial feces collection method with acid-insoluble ash as an indicator. Acid-insoluble ash content in diets and excreta was measured according to GB/T 23742-2009. Fresh excreta were collected twice daily, selecting only uncontaminated samples. After weighing, 10 mL of 10% hydrochloric acid and 3-5 drops of toluene were added per 100 g of fresh excreta, mixed thoroughly, and stored at -20°C . Energy content of all samples was determined using a PARR-6400 oxygen bomb calorimeter.

1.6 Sample Processing

Excreta processing: Excreta collected throughout the trial were pooled by replicate, dried at 65°C , equilibrated at room temperature for 24 hours, ground, and passed through a 40-mesh sieve for subsequent acid-insoluble ash and energy determination.

Carcass processing: Frozen carcasses were weighed, chopped into small pieces, and minced using a TK-22 meat grinder. After homogenization, 200 g samples were dried at 55°C for 96 hours, equilibrated at room temperature for 24 hours, reweighed, and ground for energy determination.

1.7 Calculations

NEm was determined using the regression equation recommended by Lofgreen et al. [6]: $\lg HP = a + b \times MEI$, where a and b are constants, HP is heat production, and MEI is metabolizable energy intake. NEm was estimated by fasting heat production (FHP), obtained by regressing MEI against HP for different restriction groups; when $MEI = 0$, the corresponding HP value represents FHP. NEp was calculated as final body energy minus initial body energy.

The apparent metabolizability of energy (%) was calculated as:

$$100 - 100 \times \frac{AIA_0 \times GE_i}{AIA_i \times GE_0}$$

where: - $AME = GE_0 \times$ apparent metabolizability of energy - $MEI = AME \times ADFI/BW^{0.75}$ - $HP = MEI - RE$ - $NE = NE_m + NE_p$ - Cottonseed meal $NE = (NE_i - NE_0 \times 0.85)/0.15$

AIA_0 and AIA_i represent acid-insoluble ash content in diet and feces; GE_0 and GE_i represent gross energy in feed and feces; $BW^{0.75}$ is metabolic body weight; RE is retained energy; NE_0 is NE of basal diet 2; NE_i is NE of diet with 15% cottonseed meal substitution.

1.8 Statistical Analysis

Data from NEm determination groups were analyzed using SPSS 19.0 software. One-way ANOVA was performed, and Duncan's multiple comparison test was applied when significant differences were detected. Correlation and multiple stepwise linear regression analyses were conducted between measured cottonseed meal NE values and corresponding conventional composition. Data are presented as means \pm standard deviation.

Results

2.1 Conventional Composition of Cottonseed Meal

The conventional composition of cottonseed meals used in this study is presented in Table 2, with all values conforming to the national standard "Cottonseed Meal for Feed Use" (GB/T 21264-2007).

Table 2 Conventional composition of cottonseed meal (air-dry basis) %

Items	Range	Mean±SD	CV
DM	88.72-91.4	89.80±0.68 <i>CP</i> 37.16– 50.16 43.57±3.47 <i>EE</i> 5.41– 7.88 6.18±0.46 <i>Ash</i> 0.30– 1.75 0.83±0.31 <i>CF</i> 15.22– 32.95 25.36±4.32 <i>ADF</i> 11.80– 25.19 18.19±3.10 <i>NDF</i> 10.37– 19.31 14.68±\$2.40	

2.2 Determination of NEm in Cherry Valley Ducks

As shown in Table 3, MEI and retained energy (RE) decreased significantly with increasing feed restriction levels ($P < 0.05$). Heat production (HP) also decreased significantly with restriction level ($P < 0.05$), except for the non-significant difference between 25% and 35% restriction groups ($P > 0.05$).

Table 3 MEI, RE, and HP at different feeding levels (air-dry basis) MJ/(kg $W^{0.75} \cdot d$)

Items	Ad libitum	15% restriction	25% restriction	35% restriction	45% restriction
MEI	2.39±0.96	2.15±0.90	1.96±0.79	1.80±0.67	1.66±0.57
					<i>RE</i> 1.24±0.43
					1.13±0.45 0.96±0.38 0.79±0.31

In the same row, values with different superscripts differ significantly ($P < 0.05$).

Using MEI as the independent variable and $\lg HP$ as the dependent variable, the linear regression equation was:

$$\lg HP = 0.158 \times MEI - 0.236 \quad (R^2 = 0.944, RSD = 0.000, P < 0.01)$$

When $MEI = 0$, $\lg HP = -0.236$, yielding $FHP = 580.7$ kJ/(kg $W^{0.75} \cdot d$).

2.3 NE of Substituted Diets, Cottonseed Meal, and AME-to-NE Conversion Efficiency

The NE values of substituted diets and cottonseed meal are presented in Table 4. The NE of basal diet 2 was 8.55 MJ/kg. The AME and NE of cottonseed meal were calculated using substitution values based on 15% cottonseed meal and 85% basal diet 2.

The AME-to-NE conversion efficiency for cottonseed meal was (60.97±\$2.04)%.

Table 4 NE of substituted diet and cottonseed meal and AME-to-NE conversion efficiency (air-dry basis)

Items	NEm (MJ/kg)	NEp (MJ/kg)	NE (MJ/kg)	AME (MJ/kg)	NE (MJ/kg)	AME-to-NE efficiency (%)
Range	2.81- 3.15	4.94- 5.39	8.02- 8.36	8.00- 12.22	5.04- 7.30	58.11-64.62
Mean±SD	3.97±0.10	5.22±0.12	8.19±0.09	10.05±1.02	6.12±0.62	60.97±2.04
CV (%)				10.15	10.13	

2.4 Correlation and Prediction Equations Between AME, Conventional Composition, and NE

Correlation analysis results between AME, conventional composition, and NE are shown in Table 5. NE showed the strongest correlation with AME, followed by ADF, and was also highly significantly correlated with CP, CF, and NDF ($P < 0.01$), but not significantly correlated with EE or Ash ($P > 0.05$).

Table 5 Correlation coefficients between NE and AME/conventional composition of cottonseed meal

Item	NE	AME	CP	Ash	EE	CF	ADF	NDF
NE	1							
AME	0.945**	1						
CP	0.842**	0.808**	1					
Ash	-	-	-	1				
EE	0.862**	0.793**	0.596**	0.421**	1			
CF	0.684**	0.648**	0.459**	0.808**	0.621**	1		
ADF	0.916**	0.859**	0.822**	0.793**	0.621**	0.957**	1	
NDF	0.884**	0.822**	0.793**	0.793**	0.621**	0.954**	0.979**	1
	0.862**	0.822**	0.793**					

** represents extremely significant correlation ($P < 0.01$).

Effective prediction equations for NE using AME and conventional composition are presented in Table 6. Multiple stepwise linear regression analysis selected AME, ADF, and CP as prediction factors. Among conventional components, ADF alone yielded the highest coefficient of determination (R^2) and lowest residual standard deviation (RSD). Using AME as a single predictor produced an R^2 of 0.892. Combining AME with conventional components substantially increased R^2 and decreased RSD.

Table 6 Prediction models for NE of cottonseed meal based on AME and conventional composition

Prediction models	R ²	RSD (MJ/kg)	P-value
NE = 0.402 + 0.569AME	0.892	0.21	<0.01
NE = 9.428 - 0.182ADF	0.781	0.29	<0.01
NE = -0.563 + 0.154CP	0.709	0.33	<0.01
NE = 5.209 - 0.127ADF + 0.074CP	0.823	0.27	<0.01
NE = -0.29 + 0.458AME + 0.042CP	0.923	0.17	<0.01
NE = 3.92 + 0.362AME - 0.079ADF	0.937	0.15	<0.01
NE = 3.276 + 0.241AME + 0.044CP - 0.081ADF	0.954	0.13	<0.01

Discussion

3.1 Comparison of Cottonseed Meals from Different Regions

The CP content of cottonseed meals in this study ranged from 37.16% to 50.16%. The national standard “Cottonseed Meal for Feed Use” (GB/T 21264-2007) classifies cottonseed meal into five grades based primarily on CP content (38%-50%). Our samples covered all grades specified in the standard, with other nutrients also generally compliant. The measured conventional composition was consistent with results reported by Li et al. [7], showing high coefficients of variation for all components except DM, with EE showing the greatest variation (though at low levels), followed by fiber components and CP.

3.2 Fasting Heat Production of Cherry Valley Ducks

FHP determination in this study employed the logarithmic regression model proposed by Lofgreen et al. [6], a method widely used for FHP determination in ruminants, pigs, and poultry [8-10], thus validating its application in meat ducks. The FHP of Cherry Valley ducks was measured at 580 kJ/(kg W^{0.75} · d). Previous studies reported FHP values of 598.2 [11] and 577.3 kJ/(kg W^{0.75} · d) [12] for Tianfu meat ducks. Zhang et al. [13] reported FHP values of 758 and 732 kJ/(kg BW^{0.6} · d) for growing and finishing pigs, respectively, while Zancanela et al. [14] reported 310 kJ/(kg BW^{0.6} · d) for quail. These results demonstrate substantial variation in FHP across species and even within the same species at different growth stages. De Lange et al. [15] found that regression-extrapolated FHP at zero MEI was lower than values obtained by fasting methods in growing pigs, a finding corroborated by Liu et al. [9]. The lower FHP from regression methods may result from insufficient fasting duration, where heat production from previous feed intake inflates measurements. Conversely, Gao et al. [16] reported higher FHP values from regression versus fasting methods in broilers, attributing this to stress-induced metabolic depression during fasting. These findings indicate that FHP determination methods yield different results. Additionally, NEm is affected by temperature; Sakomura

et al. [17] demonstrated that broiler NEm decreased from 497.5 to 387.0 kJ/(kg BW^{0.75}) as ambient temperature increased from 15°C to 30°C. Thus, FHP is influenced by species, growth stage, methodology, and environmental temperature. The differences between Cherry Valley and Tianfu meat ducks [11-12] can be reasonably attributed to breed variations when considering these factors.

3.3 AME, NE, and AME-to-NE Conversion Efficiency of Cottonseed Meal

Limited research exists on energy values of cottonseed meal for meat ducks, as they have long referenced broiler feed ingredient values. Zheng [18] reported a ME range of 8.06-11.75 MJ/kg (mean 9.83 ± 0.65 MJ/kg) for 19 cottonseed meals in ducks using biological methods. Li et al. [3] reported a ME range of 12.22-14.05 MJ/kg (mean 10.05 ± 1.02 MJ/kg) for 32 cottonseed meals in Cherry Valley ducks, slightly higher than previous results, possibly due to methodological differences as force-feeding may induce stress. The coefficient of variation for cottonseed meal AME was 10.15%, likely resulting from substantial variation in conventional composition.

Early cottonseed meal NE research in broilers by Fraps [19] reported NE values of 6.24-7.54 MJ/kg for CP contents of 41%-48%. Chen et al. [2] reported NE values of 5.00-7.48 MJ/kg for 25 cottonseed meals in yellow-feathered broilers, while Li et al. [3] found 4.73-7.08 MJ/kg for 15 cottonseed meals in Avian broilers, and Ning et al. [20] reported 6.35 MJ/kg for laying hens. Our results showing 5.04-7.30 MJ/kg for Cherry Valley ducks (CV = 10.13%, similar to AME) align reasonably with broiler studies, as research indicates that while chickens and ducks differ significantly in energy utilization, their utilization of cottonseed meal and corn is similar [21]. Additionally, free gossypol is a major anti-nutritional factor in cottonseed meal; Zeng et al. [22] showed that at dietary free gossypol levels below 103 mg/kg (cottonseed meal comprising 24.7% of diet), no significant effects on duck performance occurred. Our 15% substitution rate suggests gossypol did not introduce errors in NE evaluation. The NE values obtained for Cherry Valley ducks are therefore reasonable. Zinn et al. [23] reported cottonseed meal NE for dairy cattle as 7.86 MJ/kg for maintenance and 5.19 MJ/kg for production, substantially higher than values for Cherry Valley ducks. This difference likely stems from higher crude fat content (4.4%) in their cottonseed meal and cattle's superior ability to utilize fiber [24].

Our results indicate that the AME-to-NE conversion efficiency for cottonseed meal in meat ducks ranges from 58.11% to 64.62%, with variation among different cottonseed meals. Macleod [25] reported a conversion efficiency of 63% for cottonseed meal, consistent with our findings. Li et al. [11] reported $(65.93 \pm 1.85) \pm 2.00\%$ in meat ducks [26] and 78.1% in yellow-feathered broilers [27]. Swick et al. [28] demonstrated in broilers that AME-to-NE conversion efficiency increases with dietary EE content but decreases with fiber and CP content. These results confirm that high CP content reduces AME utilization efficiency. The substantial CP variation among cottonseed meals in our study resulted in lower conversion efficiency for high-protein meals due to

greater heat increment, though all values remained within reasonable ranges.

3.4 Predicting Cottonseed Meal NE Using AME and Conventional Composition

Correlation analysis revealed that fiber components were highly significantly negatively correlated with NE, while AME and CP were highly significantly positively correlated. EE and Ash showed no significant correlations with NE, likely because low EE content in cottonseed meals masked its potential positive effect. Although CP has high heat increment and is often considered a negative factor for NE, its role as the primary energy source in cottonseed meal resulted in a highly significant positive correlation with AME ($r = 0.808$), making CP a positive contributor to NE despite its higher heat increment. Numerous studies have shown that higher fiber content reduces feed effective energy, making fiber components valuable predictors. Research on predicting digestible energy of cottonseed meal in pigs identified ADF as the best predictor [29], and similar findings were reported for broiler NE prediction models [3]. Our results consistently identify ADF as the optimal conventional component predictor for cottonseed meal NE in Cherry Valley ducks. The multiple stepwise regression model achieved an R^2 of 0.954 with RSD of 0.13 MJ/kg, demonstrating high accuracy and low error for predicting cottonseed meal NE using AME and conventional composition.

Conclusion

Under the conditions of this study, the NE of cottonseed meal for 1-21 day-old Cherry Valley ducks is $(6.12 \pm 0.62) \text{ MJ/kg}$, with an AME – to – NE conversion efficiency of $(60.97 \pm 2.04)\%$. The NE of cottonseed meal correlates with its AME and conventional composition. The optimal prediction equation for Cherry Valley duck cottonseed meal NE is:

$$NE = 3.276 + 0.241AME + 0.044CP - 0.081ADF \quad (R^2 = 0.954, RSD = 0.13 \text{ MJ/kg}, P < 0.01)$$

This equation enables accurate prediction of cottonseed meal NE for Cherry Valley ducks using AME combined with conventional composition.

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Note: Figure translations are in progress. See original paper for figures.

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