

Full Length Research Paper

Levels of the amino-acid lysine in rations for broilers

Aurélio Ferreira Borges

Federal Institute of Education Science and Technology in Rondonia State, Brazil.

Received 15 February, 2017; Accepted 19 June, 2017

Synthetic amino acid supplementation has provided facilities in the adjustment of feed formula, making it possible to obtain the required levels of essential amino acids. Lysine is pronounced the second greatest restrictive amino acid in broiler nutrition. The synthetic amino acid lysine is used as the orientation amino acid in poultry for the reason that it is mainly consumed for protein synthesis. In order to characterize the requirements of lysine, 240 male broilers of the lineage Avian Farms were kept in an environment with average temperature of 25.6°C. The 22 to 42 days old broilers presented initial average weight of 541 ± 3.6 g. The basal ration contained 19.57% of crude protein (CP), 3100 kcal of metabolizable energy (ME)/kg and 0.88% of total lysine, which was supplemented with 0.000, 0.076, 0.153, 0.230 and 0.306% de L-lysine hydrochloric acid (HCL), resulting in rations with 0.88, 0.94, 1.00, 1.06 and 1.12% of total lysine. The experimental design was completely randomized with five treatments of total lysine, six repetitions and eight broilers per repetition. During experiments, the temperature was kept at 25.6 ± 0.24°C, relative moisture at 68.4 ± 6.30%, black globe temperature at 25.7 ± 0.25°C and the wet-bulb globe temperature (WBGT) at 74 ± 0.6°C. Treatments influenced the absolute weight of carcass, breast with bone, leg, thigh, abdominal fat and relative weight of breast with bone. Lysine levels had a quadratic effect over the food conversion (FC), which increased up to the level 1.03% of total lysine. Lysine levels had a quadratic influence over the weight gain (WG), which increased up to the level 1.05% of total lysine.

Key words: Chickens for meat production, growth phase, lysine, thermal environment.

INTRODUCTION

Birds, as well as mammals, are homeothermic animals, which indicated that means that even there are fluctuations at the environmental temperature, they can keep the body temperature constant (Borges et al., 2002). Any environmental change out of the thermal comfort of these animals requires behavioral, physical or physiological adjustments as attempts to adapt to the new condition. Oba et al. (2007) reported that the range

of thermal neutrality for broilers is between 24 and 28°C. Considering these adjustments, the ration intake stands out, which are reduced as temperature increases, thus causing the decrease of growth rate and worsening of food conversion (Baziz et al., 1996). However, the effect of temperature over metabolism is more complex than how it is frequently reported. According to Mendes et al. (1997), the combination of high temperature and

E-mail: aferreiraborges@yahoo.com.br.

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environment with high levels of protein reduces the growth rate and production of breast meat from broilers of the fast growth commercial lineage.

High temperatures also reduce the weight of organs in broilers, in order to reduce the metabolic rate of animals with consequent reduction of heat production. Studies conducted by Zaboli et al. (2016) showed that the protein level of broiler rations created under high temperatures may be reduced by supplementing with synthetic amino-acids without modifying their performance. Therefore, considering the different responses associated with the effect of high temperatures, it is evident that the broiler requirements vary not only due to the lineage but also in function of the thermal environment to which such animals are submitted in the different periods.

In some tropical areas of Brazil State, such as the North Region, a temperature range of 35 to 45°C from August to May is very common, and the reduction of growth performance in poultry is the most important issue. The year-on-year amplification in growth rate of modern poultry due to constant genetic improvement, global heating, and the expanding of the poultry industry in hot climates requires correct ways to lighten the consequences of heat stress. Therefore, diverse methods of growth performance in poultry have been corroborated in the world, such as climate-controlled housing, low providing density, nutritional management, lowering marketing weight, among others. Though, in greatest situations many of these practices are overpriced with low efficiency (Zaboli et al. 2016).

Protein remains the most expensive dietary nutrient, though the use of crystalline amino acids offers multiple advantages in that they provide reductions in both dietary crude protein concentrations and the excretion of dietary nitrogen into the environment (Franco et al., 2017). Belloir et al., (2017) showed that the protein level of broiler rations created under high temperatures may be reduced by supplementing with synthetic amino-acids without modifying their performance.

Lysine is considered the second most limiting amino acid in broiler diets. It is used as the reference amino acid in poultry and swine nutrition because it is mainly utilized for protein synthesis and accounts for 7.5% of carcass protein (Viola et al., 2009). The present work was conducted in order to characterize the lysine requirements for male broilers from 22 to 42 days old kept under average temperature of 25.6°C.

MATERIALS AND METHODS

Animals

The experiment was conducted in the climatic chambers of the Laboratory of Animal Bioclimatology of the Zootechny Department, Federal University of Viçosa (UFV), Brazil. Overall, 240 male broilers of the lineage Avian farms with initial average weight of 541 ± 3.6 g, vaccinated against the diseases Marek and Avian

pox. Broilers remained in the experiment from the 22nd to 42th day at high temperature (25.6°C). The experimental design was completely randomized with five treatments (levels of lysine), six repetitions and eight broilers per repetition.

Experimental diets (Table 1), isoproteic and isoenergetic, based on maize, soybean meal and corn gluten were formulated to attend the nutritional requirements of broilers regarding protein, energy, calcium, phosphorous and amino acids, except for lysine. Rations were supplemented with 0.000, 0.076, 0.153, 0.230 and 0.306% of L-lysine HCl 78.4%, resulting in rations with 0.88, 0.94, 1.00, 1.06 and 1.12% of total lysine.

Experimental design

During the initial period (1 to 21 days old), broilers were created in a conventional shed under traditional management and feeding. When 22 days were completed, broilers were weighed and transferred to climatic chambers and the experimental period started, where they remained until 42 days old. Broilers were placed in metal batteries composed by 12 compartments with area of 0.72 m² per compartment, all of them trough-type, with each compartment representing an experimental unit.

The temperature and moisture monitoring of each room was made by thermometers of maximum and minimum, dry bulb, wet bulb and black globe placed at an intermediate height in relation to the battery central compartment. Temperatures were daily recorded at two moments (8 and 18 h) during all the experiment.

The thermal environment was expressed in terms of the Wet-bulb Globe Temperature (WBGT) proposed by Buffington et al. (1981) and calculated by the following equation: $WBGT = Bgt + 0.36 Dpt - 330.08$ in which Bgt is the black globe temperature in °K and Dpt the dew point temperature in °K. Rations and water were provided at will and water was changed twice a day to avoid heating.

A continuous light program was adopted along all the experiment 24 h of artificial light by means of two fluorescent lamps of 25 Watts per room. The studied variables were: ration intake, weight gain, food conversion, total lysine intake, carcass yield, protein deposition and absolute and relative weights of prime cuts (breast, thigh and drumstick).

The calculation of ration intake during the experimental period was obtained by the difference between the amount of ration provided and lost and wastes of rations, which were weighed in the beginning and end of experiment. The weight gain of broilers was obtained by the difference between the weight in the end and beginning of experiment. The food conversion was calculated for the period from 22 to 42 days old based on data of ration intake and weight gain.

Analyses of crude protein and body composition

In the end of experiment, broilers were weighed after 12 h of fasting and posteriorly four of each repetition were chosen to be slaughtered considering the average weight of the experimental unit ($\pm 5\%$). After broilers were bled and plucked, eviscerated carcasses were weighed. Posteriorly the abdominal fat was removed and weighed. The two entire carcasses (including feet and head) of each repetition were ground during 15 min, one by one, in commercial cutter of 30 Horse Power (HP) and 1,775 rpm, and after homogenization one sample was collected. Due to the high fat content of carcasses, samples were pre-dried in forced ventilation stove at $\pm 60^\circ\text{C}$ for 72 h and pre-degreased by hot method in extractor *Soxhlet* for 4 h. After this step, samples were ground and placed in glasses for posterior evaluations. Analyses of crude protein were made in the Laboratory of Animal

Table 1. Calculated composition of the experimental diets (%).

Ingredients	Total lysine level (%)				
	0.88	0.94	1.00	1.06	1.12
Corn (7.98% PB)	65.500	65.500	65.500	65.500	65.500
Soybean meal (45.61% PB)	21.870	21.870	21.870	21.870	21.870
Corn gluten meal (60.38% PB)	7.122	7.122	7.122	7.122	7.122
Dicalcium phosphate	1.524	1.524	1.524	1.524	1.524
Limestone	1.228	1.228	1.228	1.228	1.228
Soybean oil	1.200	1.200	1.200	1.200	1.200
Salt	0.415	0.415	0.415	0.415	0.415
Mineral mix ¹	0.050	0.050	0.050	0.050	0.050
Vitamin mix ²	0.100	0.100	0.100	0.100	0.100
Butylated Toluene Hydroxide	0.010	0.010	0.010	0.010	0.010
Cocistac [*]	0.050	0.050	0.050	0.050	0.050
Choline chloride	0.125	0.125	0.125	0.125	0.125
Virginiamicin	0.055	0.055	0.055	0.055	0.055
Caulin	0.596	0.520	0.443	0.366	0.290
L-Lysine HCL (78.4%)	0.000	0.076	0.153	0.230	0.306
DL-Methionine (99%)	0.153	0.153	0.153	0.153	0.153
Tryptophan (99%)	0.005	0.005	0.005	0.005	0.005
Calculated composition					
Crude protein (%)	19.570	19.570	19.570	19.570	19.570
Metabolizable energy (kcal/kg)	3.100	3.100	3.100	3.100	3.100
Calcium (%)	0.918	0.918	0.918	0.918	0.918
Available phosphorus (%)	0.389	0.389	0.389	0.389	0.389
Sodium (%)	0.200	0.200	0.200	0.200	0.200
Total lysine	0.880	0.940	1.000	1.060	1.120
Digestible lysine (%) ³	0.780	0.840	0.900	0.960	1.020
Digestible tryptophan (%) ³	0.150	0.150	0.150	0.150	0.150
Digestible valine (%) ³	0.814	0.814	0.814	0.814	0.814
Digestible threonine (%) ³	0.632	0.632	0.632	0.632	0.632
Digestible meth + cys	0.515	0.515	0.515	0.515	0.515

¹Content/kg – Mn, 60 g; Fe, 80 g; Zn, 50 g; Cu, 10 g; Co, 2 g; I, 1 g vehicle q.s.p. 500 g. ²Content/kg - vit. A - 15,000,000 UI, vit. D3 - 1,500,000 UI, vit. E - 15,000 UI, vit. B1 - 2.0 g, vit. B2 - 4.0 g, vit. B6 - 3.0 g, vit. B12 - 0.015 g, nicotinic acid - 25 g, pantothenic acid - 10 g, vit. K3 - 3.0 g, pholic acid - 1.0 g, zinc bacitracin - 10 g, selenium - 250 mg and vehicle q.s.p. - 1,000 g. ³ Digestible amino acids calculated based on the coefficients of digestibility from Rhodimet-Rhône-Poulenc (1993) tables.

^{*}Active principle – salinomycin.

Nutrition of the Zootechny Department of Federal University of Viçosa.

An additional group of 21 days old broilers was slaughtered to determine the body composition in the beginning of experiment. The protein deposition in the carcass was calculated by the difference between the values of carcass composition between 22 and 42 days old.

The two remaining carcasses of each repetition (total of 12 carcasses per treatment) were used to obtain the prime cuts in which the absolute weight (grams) and yield (%) of entire carcasses (with feet and head), legs, thigh, drumstick, breast, feathers and abdominal fat. The carcass yield was obtained by the ratio between the weight of clean and eviscerated carcass (with feet and head) and the live weight after fasting, while the yield of prime cuts was determined considering the weight of the eviscerated carcass without feathers.

Statistical analysis

Statistical analyses were carried out through the SAEG software (1997). Estimates of total lysine requirements were established by means of linear regression and/or quadratic models and by Linear Response Plateau (LRP) according to the better adjustment.

RESULTS AND DISCUSSION

Table 2 presents the average values of environmental conditions within the climatic chambers obtained during the experimental period. The Avian Farms manual recommends the temperature around 22.5°C and moisture around 70% for the category between 22 and 42

Table 2. Average environmental conditions observed during the experimental period in the climatic chambers with broilers from 22 to 42 days old.

Variable	Values
Average air temperature (°C)	25.6±0.24
Average relative humidity (%)	68.4±6.30
Average black globe temperature (°C)	25.7±0.25
Black globe humidity index (BGHI)	73.7±0.60

Table 3. Performance, total lysine intake and protein deposition rate of male broilers from 22 to 42 days of age fed with rations containing different levels of lysine, under high temperature environment.

Parameter	Lysine levels (%)					CV (%)
	0.88	0.94	1.00	1.06	1.12	
Weight gain (g) ¹	1,219	1,265	1,293	1,315	1,283	4.53
Feed intake (g)	2,371	2,350	2,369	2,383	2,390	4.62
Feed:gain ratio ²	1.94	1.86	1.83	1.81	1.86	2.76
Total lysine intake (g) ³	21	22	24	25	27	4.81
Protein deposition rate (g)	164	166	177	180	173	3.46

^{1,2,4}Quadratic effect (P<0.09), (P<0.01) and (P<0.03), respectively. ³Linear effect (P<0.01). CV: Coefficient of variation.

days. The temperature limit of thermal-neutrality is about 25°C, and then it is possible to infer that the experimental conditions of the present work represent a moderately hot environment. A comfortable environment for such category of animals those with Black Globe Humidity Index (BGHI) around 72 and as stressing those with BGHI of 84 (Borges et al., 2002).

Table 3 presents results of performance (weight gain, ration intake and food conversion), total lysine intake and rates of fat and protein deposition in broiler carcasses from 22 to 42 days old receiving rations with different levels of lysine and kept at high temperature (25.6°C). A quadratic effect (P<0.09) was observed in the lysine levels of the ration over the weight gain (WG) of broilers up to the level 1.05%, which was associated to an intake of 25 g of total lysine. Such result was higher than 0.92 and 0.98% of total lysine obtained by Barboza (1998) for Hubbard and Ross male broilers from 22 to 40 days old, as well as to 0.85 and 1.00% of total lysine obtained by Barboza (1998) also for the weight gain of male broilers from 21 to 42 and 22 to 40 days old, respectively. On the other hand, Conhalato (1998) obtained a better result of weight gain for broilers from 22 to 42 days old created in hot periods of the year (average temperature superior to 26°C) with levels of total lysine (1.20%) higher than those found in the present work. Contrarily, Mendes et al. (1997) did not observe any influence of the lysine level over the weight gain in 22 to 42 days old broilers submitted to high environmental temperature (25.5 to 33°C).

The differences of results among the above mentioned studies may be associated to the genetic factors, as well as to the differences of environmental temperature where they were conducted. According to Cahaner et al. (1995),

the nutritional requirements of broilers are influenced by the environmental temperature and genetics. No effects of lysine levels over the ration intake (RI) were observed for the broilers. Mendes et al. (1997) worked with 21 to 42 days old broilers kept under high temperature (25.5 to 33.3°C) and also did not verify such effect. Considering that in general the animals, when exposed to high temperature, reduce the ration intake to avoid an increase of heat production (Baziz et al., 1996). it is possible to deduce that under this condition the capacity to adjust the intake is committed due to the nutrient concentration of the ration.

The lysine levels of ration had a quadratic effect (P<0.01) over the food conversion (FC), which improved up to the level 1.03% of total lysine (Table 4), what corresponded to 0.93% of digestible lysine and an estimate total lysine consumption of 24.5 g. A quadratic effect of the lysine level over the food conversion in 22 to 42 days old broilers was also recorded by Barboza (1998) and Conhalato (1998).

Levels of lysine had a quadratic effect (P<0.01) over the weight gain (WG), which improved up to 1.05% of total lysine (Table 5).

Results obtained by Trindade Neto et al. (2011) in a similar study with broilers from the same lineage and age verified that the weight variation occurred according to the equation $\hat{Y} = -1622.36 + 6602.5422X - 3019.1834X^2$, $r^2 = 86.76$ and 1.09% of lysine was indicated as optimal.

As observed for final weight, weight gain ($\hat{Y} = -2489.08 + 6596.5735X - 3028.5724X^2$, $r^2 = 83.87$) and feed conversion ($\hat{Y} = 8.55 - 12.4257X + 5.6735X^2$, $r^2 = 89.56$) also had quadratic responses (P <0.05) and estimated

Table 4. Dietary lysine level and feed:gain ratio (g/g) of broilers from 22 to 42 days old under high environmental temperature (25.6°C).

Food conversion	Total lysine of ration (%)	Ideal total lysine (%)	Quadratic equation
1.96	0.00		
1.93	0.88		
1.90	0.94		
1.87	1.00	1.03	$\hat{Y} = 7.63131 - 11.2593X + 5.45108X^2$
1.84	1.06		$r^2 = 0.98$
1.81	1.12		

r^2 = coefficient of determination.

Table 5. Weight gain (g) and lysine levels of rations for 22 to 42 days old broilers kept under high temperature (25.6°C).

Weight gain	Total lysine of ration (%)	Ideal total lysine (%)	Quadratic equation
1215	0.00		
1235	0.88		
1255	0.94		
1275	1.00	1.05	$\hat{Y} = 21.7717 - 66.5417X + 3179.67X^2$
1295	1.06		$r^2 = 0.96$
1315	1.12		

Table 6. Lysine levels of ration and ideal ration intake for the protein deposition of 22 to 42 days old broilers at high temperature (25.6°C).

Total lysine of ration	Absolute value of ration for protein deposition (g)	Linear equation
0.00		
0.88		
0.94		
1.00	25	$\hat{Y} = -1.23113 + 24.9651\text{lys}$
1.06		$r^2 = 0.99$
1.12		

the same lysine level (1.09%) as the best model. Considering that the protein deposition is more efficient than fat deposition since it aggregates more water, the increase of PDR in the carcass up to 1.05% of total lysine justifies the improvement observed for the WG and FC of broilers.

The total lysine intake increased linearly ($P < 0.01$) due to the improvement of the lysine levels in the ration according to the equation $\hat{Y} = -1.23113 + 24.9651\text{lys}$ ($r^2 = 0.99$). This result is justified by the fact that the ration intake did not vary among treatments. Although there was a linear increase of total lysine intake, the intake of 25 g provided the highest absolute values of gain and deposition of protein in the carcass (Table 6).

Regarding the carcass composition, a quadratic effect ($P < 0.03$) of the total lysine level was observed over the protein deposition rate (PDR), which increased up to the level 1.05% (Table 7). This result corroborates what was

found by Summers et al. (1992) and Deschepper and Groote (1995), who also verified alterations in the chemical composition of 42 days old broiler carcasses when low protein rations supplemented with essential amino acids in thermo-neural environments.

According to Trindade Neto et al. (2010), determining the peak of protein deposition allows estimating the maximum efficiency of amino acids used for the synthesis and accumulation of muscle mass in broiler carcasses. The effect of increase on protein deposition is characterized by the increase of water on body composition. Similarly, the reduction of body fat suggests an increase of protein synthesis efficiency and favors the accumulation of muscle mass. The deposition of protein in the carcass may be related not only to the lysine content of the diet, but also to the genetic strain and to the age of the birds utilized, as well as to thermal-environmental factors, immunologic challenge, among

Table 7. Protein deposition rate (g) and levels of lysine in rations for 22 to 42 broilers kept at high temperature (25.6°C).

PDR*	Total lysine of ration (%)	Ideal total lysine for PDR (%)	Quadratic equation
180	0.00		
176	0.88		
172	0.94	1.05	$\hat{Y} = 428.074 - 1154.76X + 550.523X^2$
168	1.00		$r^2 = 0.81$
164	1.06		
160	1.12		

*Protein deposition rate.

Table 8. Absolute and relative weights of carcass, prime cuts and abdominal fat of 42 days old broilers under high environmental temperature.

Parameter	Lysine levels					CV (%)
Absolute weight (g)	0.88	0.94	1.00	1.06	1.12	-
Weight after fasting	1,720	1,765	1,794	1,857	1,789	5.24
Carcass	1,371	1,412	1,439	1,474	1,407	5.07
Breast with bone	349	390	403	398	405	6.70
Legs	369	368	389	397	382	4.87
Drumstick	183	184	188	194	187	4.93
Thigh	186	183	202	202	193	6.38
Abdominal fat	19	16	14	16	18	16.7

others de Oliveira et al. (2013).

The lysine levels of ratio influenced ($P < 0.03$) the carcass absolute weight and yield, which increased up to the levels 1.01 and 0.96% of lysine, respectively, according to the quadratic equations $\hat{Y} = -3340.89 + 9392.72X + 4627.2X^2$, $r^2 = 0.83$ and $\hat{Y} = 27.5967 + 109.332 - 56.907X^2$, $r^2 = 0.96$ (Table 8). Contrarily, Moran Jr. and Bilgili (1990), Kidd et al. (1997) and Conhalato (1998) did not verify any influence of lysine levels over the carcass yield of 21 to 42 days old broilers created during hot months. Barboza (1998) also did not observe effect of lysine levels over the carcass yield of 22 to 40 days old broilers in thermal-neutral environment.

There was a quadratic effect of lysine levels over the absolute weight of breast with bone ($P < 0.01$), which increased up to the level 1.06% (Table 8), and a linear effect ($P < 0.01$) over the relative weight of breast with bone, which increased according to the equation $\hat{Y} = 16.81124 + 10.6134lys$ ($r^2 = 0.67$).

Barboza (1998) observed an increase of 2.8% in the breast yield as the lysine levels increased from 0.95 to 1.15% and 0.8 to 0.98%, respectively. Results obtained in the present work are coherent since according to Baker (1991) the lysine is greatly important for the composition of muscle protein. Furthermore, Moran Jr and Bilgili (1990) state that the lysine supply in adequate levels is fundamental for the production of meat

and breast. Moreover, according to Kidd et al. (1997), the requirement of essential amino acids for the maximum yield of breast is higher than what is considered adequate for the maximum growth.

The lysine levels had a quadratic effect ($P < 0.07$) over the absolute weight of legs (Table 8), which increased up to the level 1.06% of total lysine according to the equation $\hat{Y} = -497.899 + 1673.27lys - 788.216 lys^2$ ($r^2 = 0.80$). Similarly, Conhalato and Barboza (1998) did not observe effect of lysine levels over the yield of legs from 22 to 42 and 22 to 40 days old broilers, respectively. On the other hand, Mendes et al. (1997) verified an influence of increasing levels of lysine over the yield of legs from 21 to 42 days old broilers created at cyclic high temperature (25.5 to 33.3%).

Levels of lysine influenced ($P < 0.07$) the absolute weight of thigh, which had a quadratic increase up to the level 1.02% according to the equation $\hat{Y} = -713.3 - 1773.73lys - 866.164lys^2$ ($r^2 = 0.63$) (Table 8). Moreover, levels of lysine influenced ($P < 0.01$) the absolute weight of abdominal fat (Table 8), which presented a quadratic reduction up to the level 1.00% according to the equations $\hat{Y} = -315.5 - 602.109lys + 301.385lys^2$ ($r^2 = 0.98$) and $\hat{Y} = 25.6898 - 49.1016 lys + 24.4444 lys^2$ ($r^2 = 0.99$).

Mendes et al. (1997) also verified effects of the lysine levels over the abdominal fat content of 21 to 42 days old broilers created at high temperature, however, Barboza (1998) did not observe effect of levels from 0.80 to 1.10%

over the abdominal fat in 22 to 40 days old broilers when kept in neutral environment.

Conclusion

Lysine is pronounced the second greatest restrictive amino acid in broiler nutrition. The synthetic amino acid lysine it is used as the orientation amino acid in poultry for the reason that it is mainly consumed for protein synthesis. The synthetic amino acid lysine it is used as the orientation amino acid in poultry for the reason that it is mainly consumed for protein synthesis.

Lysine levels had a quadratic effect over the food conversion (FC), which increased up to the level 1.03% of total lysine. Lysine levels had a quadratic influence over the weight gain (WG), which increased up to the level 1.05% of total lysine. The total lysine intake increased linearly due to the improvement of lysine content in the ration. An effect of total lysine was observed over the protein deposition rate (PDR), which presented a quadratic increase up to the level 1.05%.

Lysine levels of the ratio influenced the absolute weight and carcass yield, which presented a quadratic increase up to the level 1.01 and 0.96%, respectively. There was a quadratic effect of lysine levels over the absolute weight of breast with bone, which increase up to the level 1.06%, and a linear effect over the relative weight of breast with bone. Lysine levels had a quadratic effect over the absolute weight of legs, which increased up to the level of 1.06%. Lysine levels influenced the absolute weight of thigh, which increased up to the level 1.02%. Lysine levels influenced the absolute weight of abdominal fat, which presented a quadratic decrease up to the level 1.00%.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest

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