

Digestible threonine-to-lysine ideal ratio for laying hens fed with low-protein diets from 24 to 40 weeks of age

Relação treonina: lisina digestíveis em rações com baixo nível de proteína bruta para galinhas poedeiras leves de 24 a 40 semanas de idade

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ABSTRACT

This trial was performed to determine the dietary digestible threonine-to-lysine (dig. Thr-to-Lys) ideal ratio for laying hens fed with low-protein diets from 24 to 40 weeks of age. At 24 weeks of age, two hundred forty Hy Line W-36 laying hens were randomly assigned to five treatments, represented by five dig Thr-to-Lys ratios (60; 67; 74; 81 and 88%), with eight replicates each one with six hens per experimental unit. Digestible Thr-to-Lys did not affect laying hen performance and egg quality, except for digestible Threonine intake, which showed linear increase as dig. Thr-to-Lys ratios increased. Linear broken-line model was chosen to analyze data according to lack of fit. According to linear broken-line estimates, digestible Threonine ideal levels for optimum egg production, egg mass, feed efficiency of lysine utilization for egg mass, feed conversion per egg mass and dozens of eggs and nitrogen balance were 0.610; 0.580; 0.614; 0.599; 0.599 and 0.643%, which correspond respectively to dig Thr-to-Lys ratios of 80; 75; 80; 78; 78 and 83%. The digestible threonine-to-lysine ideal ratio for laying hens from 24 to 40 weeks of age is 80%.

Key words: amino acids, performance, egg quality, ratios among amino acids.

RESUMO

Este estudo foi realizado para determinar a relação ideal de treonina:lisina digestíveis em rações com baixo nível de proteína bruta para galinhas poedeiras leves de 24 a 40 semanas de idade. Utilizaram-se 240 poedeiras Hy Line W-36 distribuídas em delineamento inteiramente casualizado, com cinco relações treonina:lisina digestíveis (60; 67; 74; 81 e 88%), oito repetições e seis aves por unidade experimental. As relações treonina:lisina digestíveis não influenciaram o consumo de ração e de lisina; peso médio dos ovos e seus componentes; ganho de peso e peso corporal final das aves, mas houve efeito linear para o consumo de treonina. O modelo Linear Response Plateau (LRP) foi o

que melhor representou a distribuição dos dados para taxa de postura; massa de ovos; eficiência de utilização da lisina por massa; conversão alimentar por massa e por dúzia de ovos; e balanço de nitrogênio. Os platôs ocorrem nos níveis de treonina de 0.61; 0.580; 0.614; 0.599; 0.599 e 0.643%, que corresponde às relações treonina:lisina digestíveis de 80; 75; 80; 78; 78 e 83%, respectivamente. A relação ideal de treonina:lisina digestíveis em rações para galinhas poedeiras leves de 24 a 40 semanas de idade é de 80%.

Palavras-chave: aminoácido, desempenho, qualidade de ovo, relação aminoácídica.

INTRODUCTION

Imbalance in dietary amino acids supply may implicate animal performance beyond enhancing spent of energy and amino acids, such as glycine and serine, to eliminate excessive nitrogen by poultry organism, which when excreted into environment may contaminate soil and hydric resources. The establishment of ideal amino acids profile to meet poultry physiological demand has allowed poultry nutritionist to reduce crude protein by adding crystalline amino acids to diets, which contribute to reduce nitrogen pollution into environment.

Among essential amino acids, threonine (Thr) compound the profile of amino acids demanded by poultry. Its importance in mucins synthesis in gut, antibodies and uric acid formation, besides its presence in body and egg proteins become

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threonine indispensable to maintain adequate poultry metabolism. Dietary digestible threonine and its ideal ratio with lysine optimize Thr utilization consequently resulted in optimum laying hens performance. According to SÁ et al. (2007), Thr requirement for Lohmann hens from 34 to 50 weeks of age is 583mg bird⁻¹ day⁻¹, which corresponded to the ratio with lysine in digestible basis of 70%.

As described in Brazilian Tables for Poultry and Swine, ROSTAGNO et al. (2011) suggested that dig. Thr-to-Lys ideal ratio and Thr requirement for white laying hens with egg mass of 55.50g is respectively 76% and 610mg bird⁻¹ day⁻¹; whereas, according to ROCHA et al. (2013) such ratio for Hy Line W-36 laying hens from 24 to 40 weeks of age is 77.60%.

Considering the discrepancies among published results and the importance of Thr for poultry production as well as the establishment of its ratio with lysine in digestible basis, this study was performed to determine the dig. Thr-to-Lys ideal ratio for laying hens fed with low-protein diets from 24 to 40 weeks of age.

MATERIALS AND METHODS

At 24 weeks of age, two hundred forty Hy-Line W-36 laying hens with average body weight and egg production rate of 92.80% were randomly assigned to five treatments with eight replicates each one and six hens per experimental unit. Experimental units consisted of three 25x40x45cm metal cages, where two hens were allotted, totalizing six hens. Treatments consisted of diets formulated with five digestible Thr levels (0.461; 0.515; 0.622 and 0.676%), to obtain five dig. Thr-to-Lys ratios (60; 67; 74; 81 and 88%). Dietary digestible Thr levels were obtained through the graded replacement of L-Glutamic by L-Threonine in basal diet.

Basal diet (Table 1) was formulated with 0.461% of digestible Thr, 0.768% of digestible lysine, 14.26% of crude protein. Methionine + cysteine, tryptophan, isoleucine, valine, arginine, leucine, histidine and phenylalanine + tyrosine-to lysine ratios in digestible basis considered to formulate basal diet were respectively 103; 28; 76; 96; 103; 152; 44 and 137%. Dietary digestible lysine was supplied in 90% of those recommended by ROSTAGNO et al. (2011). Methionine + cysteine, tryptophan, isoleucine and valine-to lysine ratios were met in 103% of those recommended by BRUMANO et al. (2010), CALDERANO et al. (2012), MELLO et al.

(2012) and ALMEIDA (2014) respectively. Ratios of the other essential amino acids with lysine in digestible basis were supplied, at least, 3% above the recommended by ROSTAGNO et al. (2011). Such criteria in amino acids supply aimed to guarantee the maximum utilization of lysine and to avoid dietary amino acids deficiency. Thus the ratio between amino acids will represent hen requirement.

All the other nutrients in diets, except Thr and crude protein, were supplied according to ROSTAGNO et al. (2011) nutritional recommendations, considering hen average feed intake of 95g bird⁻¹. Diets were daily supplied at 8 and 16h. During the experimental trial, hens had free access to diets and water. Lighting program consisted of supply of 17h of light a day. Temperature inside the experimental facility were recorded at 16h, by two thermometers birds-height placed.

Variables assessed were: egg production; egg weight; egg mass; feed intake; threonine and lysine intake; efficiency of lysine utilization per egg mass produced; feed conversion per egg mass and dozens of eggs; albumen, yolk and eggshell weight and percentage; final body weight and body weight gain; and nitrogen balance.

Nitrogen balance was estimated through excreta total collection. At 40th week, excreta samples from four replicates of each treatment were collected during three days and stored at freezer. Excreta and experimental diets nitrogen content were analyzed according to Kjeldahl method description. Nitrogen balance (%), expressed in dry matter was obtained through division of total nitrogen retained by total nitrogen ingested.

After analyses of variance (ANOVA) data were analyzed using polynomial and/or linear broken-line regression. Data were analyzed using R software package (R Development Core Team, 2009) and significant effects were considered when $P < 0.05$.

RESULTS AND DISCUSSION

Minimum and maximum temperature recorded inside the experimental facility during the trial was respectively 19±2 and 31±2°C. According to Hy-Line W-36 guideline (HY LINE W36, 2015), the hen thermal comfort zone is 18-25°C. Thus, considering the temperature recorded inside the facility, the hens were exposed to periods of heat stress.

Laying hen performance was affected by dig. Thr-to-Lys ratios (Table 2). Egg production showed a liner and linear broken-line response as dig. Thr-to-Lys ratios increased according to regression equations:

Table 1 - Composition and nutritional value of experimental diets.

Ingredients (%)	-----Digestible Thr-to-Lys ratio (%)-----				
	60	67	74	81	88
Corn	65.793	65.793	65.793	65.793	65.793
Soybean meal (45%)	18.034	18.034	18.034	18.034	18.034
Limestone	10.226	10.226	10.226	10.226	10.226
Soybean oil	2.405	2.405	2.405	2.405	2.405
Dicalcium phosphate	1.280	1.280	1.280	1.280	1.280
Salt	0.555	0.555	0.555	0.555	0.555
Potassium carbonate	0.161	0.161	0.161	0.161	0.161
Choline chloride	0.020	0.020	0.020	0.020	0.020
Vitamin premix ¹	0.100	0.100	0.100	0.100	0.100
Mineral premix ²	0.050	0.050	0.050	0.050	0.050
Antioxidant ³	0.010	0.010	0.010	0.010	0.010
DL- methionine (99%)	0.404	0.404	0.404	0.404	0.404
L-lysine HCl (78%)	0.232	0.232	0.232	0.232	0.232
L-tryptophan (98%)	0.083	0.083	0.083	0.083	0.083
L-valine (98%)	0.166	0.166	0.166	0.166	0.166
L-isoleucine (99%)	0.081	0.081	0.081	0.081	0.081
L-threonine (98%)	0.000	0.060	0.119	0.178	0.237
L-glutamic (99%)	0.395	0.312	0.231	0.150	0.068
Inert ⁴	0.005	0.028	0.050	0.072	0.095
Total	100.000	100.000	100.000	100.000	100.000
	-----Calculated composition-----				
Crude protein (%)	14.260	14.260	14.260	14.260	14.260
Metabolizable energy (kcal/Kg)	2,900	2,900	2,900	2,900	2,900
Calcium (%)	4.232	4.232	4.232	4.232	4.232
Non-phytate phosphorous (%)	0.316	0.316	0.316	0.316	0.316
Sodium (%)	0.237	0.237	0.237	0.237	0.237
Potassium (%)	0.611	0.611	0.611	0.611	0.611
Dig. lysine (%)	0.768	0.768	0.768	0.768	0.768
Dig. methionine + cysteine (%)	0.791	0.791	0.791	0.791	0.791
Dig. arginine (%)	0.796	0.796	0.796	0.796	0.796
Dig. phenylalanine + tyrosine (%)	1.055	1.055	1.055	1.055	1.055
Dig. histidine (%)	0.340	0.340	0.340	0.340	0.340
Dig. leucine (%)	1.168	1.168	1.168	1.168	1.168
Dig. isoleucine (%)	0.584	0.584	0.584	0.584	0.584
Dig. threonine (%)	0.461	0.515	0.569	0.622	0.676
Dig. tryptophan (%)	0.215	0.215	0.215	0.215	0.215
Dig. valine (%)	0.737	0.737	0.737	0.737	0.737

¹Vitamin premix – Composition kg⁻¹: vit. A - 7.200.000U.I. vit. D₃ - 1.600.000U.I. vit. E - 5.000mg. vit. B₁ - 900mg. vit. B₂ - 2.700mg. vit. B₆ - 1.500mg. vit. B₁₂ - 7.200mg. pantothenic acid - 5.900mg. vit. K₃ - 1.100mg. folic acid - 250mg. niacine - 16.200mg. selenium - 250mg. antioxidant additive - 250mg; ²Mineral premix- Composition kg⁻¹: manganese - 100.000mg. iron- 60.000mg. zinc - 800.000mg. copper - 12.000mg. iodine - 1.000mg. selenium - 300mg; ³tert-butyl-4-hydroxy toluene (BHT); ⁴sand.

Table 2 - Performance and nitrogen balance of White laying hens from 24 to 40 weeks of age fed different digestible Thr-to-Lys ratios.

Variable	-----Digestible Thr-to-Lys ratio (%)-----					CV (%)	P-value
	60	67	74	81	88		
Egg production (%) ^{1,2}	90.81	91.80	92.32	94.32	93.79	2.09	0.005
Egg weight (g)	59.47	59.80	59.23	59.63	58.50	2.31	0.725
Egg mass (g bird ⁻¹ day ⁻¹) ^{1,2}	53.96	54.84	54.79	56.23	55.43	2.23	0.012
Feed intake (g bird ⁻¹ day ⁻¹)	94.13	93.98	91.98	93.03	92.05	2.47	0.200
Threonine intake (mg bird ⁻¹ day ⁻¹) ¹	433.95	483.99	523.36	578.66	622.25	2.39	<0.001
Lysine intake (mg bird ⁻¹ day ⁻¹)	722.94	721.76	706.37	714.49	706.93	2.47	0.200
Efficiency of lysine utilization per egg mass (g g ⁻¹) ^{1,2}	74.67	75.99	77.75	78.76	78.23	2.10	<0.001
Feed conversion (kg dozen ⁻¹) ^{1,2}	1.25	1.23	1.20	1.18	1.18	2.92	0.001
Feed conversion (kg kg ⁻¹) ^{1,2}	1.75	1.72	1.68	1.66	1.67	1.98	<0.001
Body weight gain (g bird ⁻¹)	54	88	78	90	69	44.75	0.213
Final body weight (kg bird ⁻¹)	1.50	1.54	1.52	1.55	1.52	2.23	0.098
Nitrogen balance (%) ^{1,2}	53.98	55.88	58.06	59.01	62.65	5.79	0.024

CV=coefficient of variation; 1=linear effect; 2=linear broken-line effect.

$\hat{Y} = 83.6453 + 15.7605x$, $r^2 = 86.49$ e $\hat{Y} = 81.1851 + 20.5386x$, $r^2 = 92.96$, respectively. According to linear broken-line equations, the digestible Thr level from which the plateau was observed was 0.613%, which corresponds to dig. Thr-to-Lys ratio of 80% and egg production rate of 93.78%.

Digestible Thr-to-Lys ideal ratio estimated for optimum egg production (80%) in this trial was greater than the one determined by ROCHA et al. (2013), who established the dig. Thr-to-Lys ratio of 78% as ideal for Hy-Line W-36 laying hens at the same age. The greater Thr need reported in this trial compared to those observed by ROCHA et al. (2013) may be justified by the higher egg production rate observed in this trial (93.78%) compared to the authors (84.42%). The average egg production in this trial was 92.60%, which is approximately 1% inferior to the recommended by the genetic line guideline (HY LINE W36, 2015). Although the difference in egg production is narrow, it is important to observe that experimental diets were formulated to provide a sub-marginal digestible lysine supply, once this trial was performed to determine the dig. Thr-to-Lys ideal ratio for optimum laying hen performance and egg quality; and the establishment of such ratio and digestible lysine requirement allowed to determine ideal digestible Thr level in diets.

Egg weight was not influenced ($P>0.05$) by dietary dig. Thr-to-Lys ratios. Linear and linear broken-line ($P<0.05$) regression effects was verified for egg mass production as dietary dig. Thr-to-Lys increased, as described by the following regression equations: $\hat{Y} = 50.4717 + 8.05188x$, $r^2 = 66.28$ e \hat{Y}

$= 48.1656 + 12.5306x$, $r^2 = 85.87$, respectively. The plateau was estimated from dig. Thr-to-Lys ratio of 75%, which corresponded to egg mass value of 55.43g bird⁻¹ day⁻¹. The increasing observed in egg mass values in dig. Thr-to-Lys are expected, once egg production increased, whereas egg weight was not affected by diets (Table 2). Similarly, SÁ et al. (2007) and LELIS (2010) did not observe effects of dig. Thr-to-Lys ratios on egg weight. However, authors reported dig. Thr-to-Lys optimum ratios for egg mass of 72 and 72.7% respectively.

The dig. Thr-to-Lys ratio of 75% is similar to those described by ROSTAGNO et al. (2011), which recommended the dig. Thr-to-Lys of 76% for laying hens with egg mass production of 55g bird⁻¹ day⁻¹. However, comparing the Thr requirement determined in this trial (535mg bird⁻¹ day⁻¹) with those described by ROSTAGNO et al. (2011) (610mg bird⁻¹ day⁻¹), it is observed that hens in the current trial presented higher efficiency, once they presented lower feed intake (75mg bird⁻¹ day⁻¹ less Thr) and produced equivalent egg mass (55g ave⁻¹ dia⁻¹).

Average daily intake in this trial was 93g bird⁻¹, which close to feed intakes recommended by genetic line guideline (92g bird⁻¹ day⁻¹) (HY LINE W36, 2015). Thus, heat stress during the trial did not affect feed intake. Digestible Thr-to-Lys ratios did not influence hen feed intake ($P>0.05$). Such result is in agreement with published reports (SÁ et al., 2007; LELIS, 2010) and indicated that laying hens are not capable of regulating Thr deficiency through enhancing in feed intake (MARTÍNEZ-AMESCUA et al., 1999).

Threonine intake enhanced linearly ($P < 0.05$) as dietary dig. Thr-to-Lys ratio increased, as demonstrated by the following regression equation: $\hat{Y} = 29.5080 + 877.481x$, $r^2 = 99.78$. Such behavior of response is justified by linear increasing in dietary Thr supply to obtain dig. Thr-to-Lys ratios; and no effects of dig. Thr-to-Lys ratios on laying hen feed intake.

Digestible lysine intake was not affected by experimental diets ($P > 0.05$), which is expected, once lysine in experimental diets, were equally supplied and feed intake was not influenced by dig. Thr-to-Lys ratios. However, it was observed linear and linear broken-line ($P < 0.05$) effects on efficiency of lysine utilization per egg mass, as described by regression equations: $\hat{Y} = 66.5893 + 18.4495x$, $r^2 = 84.47$ e $\hat{Y} = 62.6163 + 26.1657x$, $r^2 = 99.13$, respectively. The plateau in laying hen responses was observed from Thr level of 0.614% on, which corresponds to dig. Thr-to-Lys ratio of 80% and the value of 78.23g g^{-1} .

The efficiency of lysine utilization per egg mass is an important measure, once it isolates the effect of the amino acids studied on hen performance, specifically when treatments influence feed intake and lysine intake. Such variable allows identifying in which treatment the lower amount of lysine was used to produce one gram of egg mass. In the current trial, the greater efficiency of lysine utilization was obtained with the dig. Thr-to-Lys ratio of 80%.

Feed conversion per egg mass (kg kg^{-1}) and per dozen (kg dozen $^{-1}$) reduced linearly ($P < 0.05$) when dig. Thr-to-Lys ratios increased, as described by the regression equations: $\hat{Y} = 1.92623 - 0.409749x$, $r^2 = 85.14$ e $\hat{Y} = 1.39048 - 0.323566x$, $r^2 = 85.84$; respectively. Linear broken-line ($P < 0.05$) effect of dig. Thr-to-Lys ratios was observed on feed conversion per egg mass (kg kg^{-1}) and per dozen (kg dozen $^{-1}$), as described by the equations: $\hat{Y} = 62.6163 + 26.1657x$, $r^2 = 99.13$ e $\hat{Y} = 1.45401 - 0.446944x$, $r^2 = 97.99$. The plateau for feed conversion per egg mass and per dozen of eggs were both obtained from the dig. Thr-to-Lys ratio of 78%, which corresponds to Thr level of 0.599% and which resulted in feed conversion per egg mass and dozen of eggs values of 1.66kg kg^{-1} and 1.18kg dz^{-1} , respectively.

ROCHA et al. (2013) observed improvement in feed conversion per egg mass and dozen of eggs values with dig. Thr-to-Lys ratios of 75 e 77.60%, respectively. SA et al. (2007) verified better values of feed conversion per dozen of eggs with dig. Thr-to-Lys ratio of 72%. Although the lower dig. Thr-to-Lys ratio observed by the authors,

compared with the ideal ratio determined in the current trial (78%), the Thr intake estimated by SA et al. (2007) was greater than the observed in this trial (583 x 555mg bird $^{-1}$). Such difference is due to bird daily feed intake, which in this trial was 93g bird $^{-1}$, whereas SÁ et al. (2007) observed 114g bird $^{-1}$. In both trials, dig. Thr-to-Lys ideal ratio were determined with white laying hens.

The dig. Thr-to-Lys ratios was not observed ($P > 0.05$) on final body weight and hen weight gain (Table 2). However, the hens were fed with diets containing sub-marginal level of lysine, which could have influenced weight gain and final body weight. Linear and linear broken-line effects of dietary dig. Thr-to-Lys ratios ($P < 0.05$) were observed on nitrogen balance (Table 2), as described by the equations: $\hat{Y} = 36.2349 + 38.1268x$, $r^2 = 96.90$ e $\hat{Y} = 36.5098 + 37.789x$, $r^2 = 99.84$, respectively. The plateau was established from the Thr level of 0.643%, which corresponds to dig. Thr-to-Lys ratios of 83.70% and nitrogen balance of 60.82%.

Similar behavior was observed in nitrogen balance and egg production responses. The plateau for egg production rate was established from the dig. Thr-to-Lys ratio of 80%; whereas, the same response for nitrogen balance was observed from the ratio of 83.70%. Thus, it was possible to conclude that greater egg production rate requires greater nitrogen amount to maintain egg protein synthesis, which is obtained from dietary supply. Thus, less nitrogen is excreted, which impacts positively in nitrogen balance.

MATOS et al. (2009) did not observe influence of dietary Thr levels (0.50; 0.55 e 0.60%) on laying hen nitrogen balance at 44 weeks of age, as well as on hen egg production from 25 to 44 weeks of age. Digestible Thr-to-Lys ratios did not affect ($P > 0.05$) albumen, yolk and eggshell weight and percentage (Table 3). Such results are in agreement with those observed in egg weight. Thus, the dig. Thr-to-Lys ratio of 60%, which corresponds to digestible Thr intake of 434mg bird $^{-1}$, maintaining adequate egg quality of white laying hens from 24 to 40 weeks of age.

ROCHA et al. (2013), as well as observed in this trial, did not observe effects of dietary dig. Thr-to-Lys ratios on albumen, yolk and eggshell weight and percentage for white laying hens from 24 to 40 weeks of age. Though KAKIMOTO et al. (2014) who studied dig. Thr-to-Lys ratios for brown laying hens from 26 to 42 weeks of age, observed that digestible Thr levels of 0.65 and 1.1% resulted in greater and lower yolk percentage and lower and greater albumen percentage respectively.

Table 3 - Egg quality of laying hens from 24 to 40 weeks of age fed with different digestible Thr-to-Lys ratios.

Variables ¹	-----Digestible Thr-to-Lys ratio (%)-----					CV (%)	P-value
	60	67	74	81	88		
Albumen (g)	38.79	39.05	38.30	38.74	38.40	0.76	0.383
Yolk (g)	15.74	15.85	15.70	15.56	15.58	2.00	0.501
Eggshell (g)	5.55	5.61	5.48	5.60	5.45	2.58	0.386
Albumen (%)	64.59	64.56	64.42	64.72	64.60	0.76	0.827
Yolk (%)	26.18	26.17	26.38	25.93	26.21	2.00	0.583
Eggshell (%)	9.23	9.27	9.21	9.35	9.19	2.58	0.678

¹Non-significant; CV=coefficient of variation.

CONCLUSION

The dig. Thr-to-Lys ideal ratio for laying hens fed with low-protein diets from 24 to 40 weeks of age is 80%, which corresponds to the threonine and lysine intake of 571mg bird⁻¹ day⁻¹ and 713mg bird⁻¹ day⁻¹ respectively.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

Approved by the ethics commission in use of production animals of Universidade Federal de Viçosa (UFV), under protocol n. 24/2014.

ACKNOWLEDGMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship granted and Ajinomoto Biolatina Indústria e Comércio Ltda for the financial support.

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