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## Digestible threonine:lysine ratios for light laying hens from 29 to 45 weeks

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#### **ABSTRACT**

The objective was to determine the nutritional requirement of digestible threonine and the threonine: lysine ratios for light laying hens from 29 to 45 weeks of age. Two hundred Hy-Line-36® hens were distributed in a completely randomized design with five replicates and 10 birds per experimental unit and four treatments (5.50, 5.77, 6.05 and 6.30 g digestible threonine kg<sup>-1</sup>), establishing threonine: lysine of 69, 73, 76 and 80%. Four production cycles of 28 days each were evaluated: feed intake, feed conversion (kg dz<sup>-1</sup>), feed conversion (kg kg<sup>-1</sup>), egg laying percentage, weight and egg mass, percentage of egg components, internal egg quality and shell thickness. No effect of threonine levels was observed on egg production, weight and egg mass, internal egg quality, albumen and yolk percentage, specific gravity, shell thickness, feed intake, feed conversion (kg dz<sup>-1</sup>) and feed conversion ratio (kg kg<sup>-1</sup>). The ratio of digestible threonine: lysine to light laying hens in the period from 29 to 45 weeks is 69%, which corresponds to a daily consumption of 520 mg threonine bird<sup>-1</sup> day<sup>-1</sup>.

Key words: amino acid; performance; egg production; internal quality

# Relações treonina: lisina digestíveis para poedeiras leves no período de 29 a 45 semanas

#### **RESUMO**

Objetivou-se determinar a exigência nutricional de treonina digestível e as relações treonina: lisina para poedeiras leves no período de 29 a 45 semanas de idade. Duzentas aves Hy-Line—36® foram distribuídas em delineamento inteiramente casualizado com cinco repetições e 10 aves por unidade experimental e quatro tratamentos (5,50; 5,77; 6,05 e 6,30 g de treonina digestível kg-1, estabelecendo a relação treonina: lisina de 69, 73, 76 e 80%. Foram estudados, quatro ciclos de produção de 28 dias cada, sendo avaliados: consumo de ração, conversão alimentar (kg dz-1), conversão alimentar (kg kg-1), percentual de postura, peso e massa dos ovos, porcentagem dos componentes dos ovos, qualidade interna dos ovos e espessura de casca. Não foi observado efeito dos níveis de treonina sobre o percentual de postura, peso e massa de ovos, qualidade interna dos ovos, porcentagem de albúmen e gema, densidade específica, consumo de ração, conversão alimentar (kg dz-1) e conversão alimentar (kg kg-1) e espessura de casca. A relação de treonina: lisina digestíveis para poedeiras leves no período de 29 a 45 semanas é de 69%, que corresponde um consumo diário de 520 mg treonina-1 ave-1 dia-1.

Palavras-chave: aminoácido; desempenho; produção de ovos; qualidade interna

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## Introduction

With genetic advances, commercial laying hens are increasingly precocious and with higher production peaks, indicating that their nutritional requirements need to be revised to ensure maximum performance associated with increased bird genetic progress over the last fifteen years (Bregendahl et al., 2008). On the other hand, the availability of synthetic amino acids have enabled the nutritionist to formulate feeds more adjusted to meet the requirement of the birds, avoiding excessive amino acids and reducing costs.

According to Schmidt et al. (2011), the formulation of feeds based on amino acid levels is a widely used concept in poultry nutrition with the aim of reducing the crude protein content in the rations. For Figueiredo et al. (2012), the essential amino acid levels in laying rations have important influence on egg size, albumen and yolk deposition, percentage of total solids and internal quality of eggs. Among the amino acids, threonine, is considered essential in diets based on corn and soybean meal, being the third limiting amino acid to be supplemented in feed for laying hens, after lysine and methionine (Matos et al., 2009; Cupertino et al., 2010).

Threonine becomes more important with the advancement of the age birds, since the requirement proportion of threonine for maintenance is high, mainly during the peak of production, a phase in which the nutrient demand is greater. Thus, supplementation of threonine in adequate form allows the bird to express its maximum egg production potential (Jordão Filho et al., 2006). According to Sá et al. (2007), the nutritional requirement of threonine during this period should be considered, because this amino acid is indirectly required in egg formation.

Yet, the amino acid threonine participates in the synthesis and maintenance of the body protein, acting in the production of antibodies and gastrointestinal mucin and playing an important role as precursor of glycine and serine and generating many important catabolic products in the metabolism (Azzam et al., 2014). Threonine levels should be in adequate amounts also due to their wide participation in the body and in animal metabolism.

According to Duarte et al. (2012), formulation of minimum cost diets meeting the requirement of threonine is essential to decrease amino acid imbalance, since threonine deficiency may reduce the efficiency of use of other limiting amino acids. Moreover, excessive amino acids may compromise the performance of laying hens due to increased amounts in the bloodstream. This excess of amino acids is be metabolized with energy expenditure in which the anima group is diverted to the formation of uric acid, having its volume increased in the bloodstream of birds (Agustini et al., 2014).

Thus, studies about the relationship between the digestible amino acids threonine and lysine are necessary, since few studies have addressed the nutritional requirement of threonine and the threonine:lysine ratio for light laying hens. The aim of this study was to determine the digestible threonine:lysine ratio for lightweight laying hens aged 29 to 45 weeks.

## **Material and Methods**

The experiment was carried out in the Aviculture sector of the Goiano Federal Institute - Câmpus Ceres and approved by the Ethics and Research Committee of the Federal Institute of Goiás with protocol number 017/2012, in accordance with current legislation and the Ethical Principles published by the Brazilian College of Animal Experimentation (COBEA).

A total of 200 lightweight hens with a mean initial weight of 1.424 kg of commercial Hy-Line 36® strain were used in the period from 29 to 45 weeks of age, distributed in a completely randomized design with four treatments, five replicates and ten birds per experimental unit. In the breeding, rearing and production phases, the birds were managed as described in the lineage manual. Temperature and relative humidity were monitored daily, with maximum and minimum averages of 31.6°C and 16.7°C, respectively, and mean air relative humidity of 76.6%. The light program was constant with 16 hours of light, remaining throughout the experimental period. The experimental birds were selected at the 27th week of age, standardized according to body weight and distributed in cages (25 cm wide, 44.5 cm long and 42 cm deep) to verify feed intake.

A basal diet formulated with corn and soybean meal was prepared, using starch as inert, to evaluate the nutritional requirement of digestible threonine (Table 1).

The basal diet met the nutritional recommendations proposed by the strain manual, with 15.74% crude protein and

Table 1. Composition and nutritional value of experimental diets.

Inquadiants (a leaf)	Relations (%)				
Ingredients (g kg-1)	69	73	76	80	
Corn	569.81	569.81	569.81	569.81	
Soybean Bran 45%	240.72	240.72	240.72	240.72	
Limestone	100.48	100.48	100.48	100.48	
Soy oil	35.00	35.00	35.00	35.00	
Phosphate Bicalcium	22.67	22.67	22.67	22.67	
Wheat Bran	10.00	10.00	10.00	10.00	
Common Salt	4.37	4.37	4.37	4.37	
Premix*	4.00	4.00	4.00	4.00	
DL-Methionine	2.23	2.23	2.23	2.23	
L-Lysine HCI (79%)	0.74	0.74	0.74	0.74	
L-Threonine (98%)	0.14	0.41	0.70	0.97	
Starch	10.00	9.59	9.30	9.03	
Total	1.000	1.000	1.000	1.000	
Nutritional composition (g kg -1)					
Crude Protein	157.4	157.4	157.4	157.4	
Metabolizable energy (Mcal kg <sup>-1</sup> )	2.844	2.844	2.844	2.844	
Calcium	44.2	44.2	44.2	44.2	
Phosphorus available	5.1	5.1	5.1	5.1	
Lysine digestible	7.90	7.90	7.90	7.90	
Digestible methionine	4.38	4.38	4.38	4.38	
Digestible threonine	5.50	5.77	6.05	6.30	
Methionine + digestible cystine	6.6	6.6	6.6	6.6	
Tryptophan digestible	1.7	1.7	1.7	1.7	
Arginine digestible	3.07	3.07	3.07	3.07	
Isoleucine digestible	6.02	6.02	6.02	6.02	
Linoleic acid	3.163	3.163	3.163	3.163	
Chlorine	3.07	3.07	3.07	3.07	
Sodium	1.9	1.9	1.9	1.9	

\*vit. A: 1,750.000 ui kg-¹, vit. D3 500,000 ui kg-¹, vit. E: 1,250 ui kg-¹, vit. K3: 400 mg kg-¹, vit. B: 750 mg kg-¹, vit. B12: 2,000 mg kg-¹, niacin: 5,000 mg kg-¹; Choline: 58.590 g kg-¹, Pantothenic Ac.: 1,250 mg kg-¹, methionine: 247.50 g kg-¹, copper: 2,000 mg kg-¹, iron: 12.500 g kg-¹, manganese: 17.500 g kg-¹, zinc: 12.500 g kg-¹, lodine: 300 mg kg-¹, selenium: 50 mg kg-¹, bacitracin Zn: 5,000 mg kg-².

0.550% digestible threonine. The other levels studied were obtained with additions of L-Threonine to replace the starch of the basal diet, maintaining the isoenergetic and isoprotein diets, in order to meet the following proposed levels: 5.50; 5.77; 6.05; and 6.30 g digestible threonine kg<sup>-1</sup>, yielding the threonine:lysine ratio of 69%, 73%, 76% and 80%, respectively. The feeds were provided daily in two periods, assuring ad libitum consumption of feed and water throughout the experimental period.

The experimental trial was divided into four production periods with duration of 28 days. At the end of each cycle, feed intake, feed conversion (kg dz<sup>-1</sup>), feed conversion (kg kg<sup>-1</sup>), laying percentage, egg weight, egg mass, percentage of egg components (albumen, shell and yolk), internal egg quality (Haugh unit, albumen and yolk index) were assessed. In relation to the percentage of egg components and internal quality, four eggs per experimental unit were collected during the last three days of each 28-day period.

Eggs components were obtained after weighing each egg and then breaking them, thus obtaining the yolk and the shell weight. Shells were weighed after being washed in tap water and dried at room temperature for 48 hours. The weight of the albumen was determined by subtracting the difference between the weight of the egg and of the shell and the yolk.

In order to determine the Haugh unit, eggs were weighed and then broken to measure the albumen height with the aid of a digital caliper; the Haugh unit was calculated using the following formula: UH = 100 Log (h - 1.7 w + 7.6), where: UH = Haugh unit; H = dense albumen height (mm); w = egg weight (g), according to Cotta (1997). To determine albumen and yolk indices, the height and diameter of albumen and yolk were measured using a digital caliper. Egg production was computed daily according to the number of birds housed per experimental unit to obtain the egg laying percentage. The methodology of Moreng & Avens (1990) was used to assess the specific egg density, which consists of immersing the eggs in buckets with different salt solutions, with densities ranging from 1.060 to 1.100 g cm<sup>-3</sup>. Shell thickness was determined using a digital caliper, measured at two different points and the result is expressed in mm.

Data were submitted to analysis of variance and polynomial regression in the statistical program SAS (2000).

## **Results and Discussion**

The production, weight and egg mass were not influenced (P < 0.05) by threonine levels in the diet (Table 2). This suggests that for the maximum production, weight and mass

of eggs of lightweight laying hens at the peak of production, the nutritional requirement of digestible threonine should be 5.50 g kg<sup>-1</sup>, with a digestible threonine:lysine ratio of 69%, confirming the recommendations made by the Hy-Line-36® strain manual. However, Rostagno et al. (2011) predicted the threonine:lysine ratio of 76% for lightweight laying hens, without strain specification.

Figueiredo et al. (2012) found no effect of threonine levels on egg production, weight and mass of eggs of lightweight laying hens from 42 to 58 weeks of age and concluded that the requirement for this amino acid is equal to or less than the lowest level studied, which was 0.542%, close to the present results.

On the other hand, Schmidt et al. (2011) found that the production, weight and mass of eggs were influenced by quadratic levels of threonine in the diet. The threonine requirement for these variables was estimated to be 0.465, 0.445 and 0.459%, corresponding to a daily intake of 455, 433 and 448 mg of threonine bird<sup>-1</sup> day<sup>-1</sup>, respectively. In the present study, the daily intake of digestible threonine was 520, 542, 569 and 605 mg of threonine bird<sup>-1</sup> day<sup>-1</sup>.

In relation to the egg mass, Ishibashi et al. (1998) determined the nutritional requirement of threonine of 455 mg bird-1 day-1 for maximum egg mass production of commercial laying hens from 29 to 39 weeks of age, unlike the results found by Bregendahl et al. (2008) of 414 mg bird-1 day-1 in the 26 to 34 week period for Hy-Line W-36 laying hens, with a threonine:lysine ratio corresponding to 77% for maximum egg mass.

Faria et al. (2002) observed a linear response of egg production and egg mass in relation to threonine levels in the diet of lightweight laying hens. The daily requirement of threonine for the egg production corresponded to 439 mg bird<sup>-1</sup> day<sup>-1</sup> and to the egg mass, 461.1 mg bird<sup>-1</sup> day<sup>-1</sup>, for laying hens from 31 to 38 weeks of age.

Feed intake, feed conversion per dozen eggs and kg of egg were not influenced (p < 0.05) by the levels of threonine in the diet (Table 2). Likewise, Azzam et al. (2014) also found no effect of threonine levels on the feed consumption of lightweight laying hens, agreeing with the result obtained in this study. In their study, the variable threonine:lysine ratio was 69%. On the other hand, Sá et al. (2007) found a quadratic effect of the feed conversion per dozen eggs in relation to the levels of digestible threonine studied in diets of lightweight laying hens in the period from 34 to 50 weeks of age.

There was no effect (P < 0.05) on the percentage of albumin and yolk, index of yolk and albumen and Haugh unit (Table 3). These results are in agreement with the ones presented by

Table 2. Egg production, weight and mass, feed intake and feed conversion (kg kg<sup>-1</sup> and kg dz<sup>-1</sup>), of lightweight laying hens fed different digestible threonine:lysine ratios, from 29 to 45 weeks.

Threonine levels	Threonine:lysine ratios	Egg production	Egg weight	Egg mass	Feed intake	Feed conversion	Feed conversion
(g kg <sup>-1</sup> )	(%)	(%)		$(g^{-1} bird^{-1} day^{-1})$		(kg of feed egg kg <sup>-1</sup> )	(feed kg dozen egg <sup>-1</sup> )
5.50	69	89.08	58.15	53.04	94.46	1.791	1.246
5.77	73	91.58	59.41	55.55	94.02	1.695	1.207
6.05	76	89.64	59.08	53.86	94.11	1.759	1.246
6.30	80	90.08	60.78	55.53	96.06	1.734	1.264
Coefficier	nt of variation (%)	5.16	2.91	5.38	5.51	5.86	4.74
	P value	0.85	0.161	0.458	0.917	0.513	0.500

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Table 3. Percentage of albumin and yolk, yolk index and Haugh unit in lightweight laying hens aged 29 to 45 weeks and fed with different digestible threonine:lysine ratios.

Threonine levels (g kg <sup>-1</sup> )	Threonine:lysine ratios	% albumen	% of yolk	Yolk index	Albumen index	Haugh unit
5.50	69	61.37	28.71	0.426	0.167	103.70
5.77	73	61.28	29.30	0.432	0.168	103.89
6.05	76	61.01	29.60	0.426	0.164	104.51
6.30	80	62.37	28.88	0.426	0.167	103.84
Coefficient	of variation (%)	2.05	3.78	1.68	2.11	0.73
P value		0.373	0.584	0.481	0.512	0.861

**Table 4.** Percentage of shell, shell thickness and specific density of lightweight laying hens fed with different ratios of digestible threonine, in the period from 29 to 45 weeks.

Threonine levels (g kg <sup>-1</sup> )	Threonine:lysine ratios	% Shell	Shell thickness (mm)	Specific gravity (g cm <sup>-3</sup> )
5.50	69	9.90ª	0.377	1.093
5.77	73	9.64 <sup>b</sup>	0.379	1.093
6.05	76	9.70ab	0.377	1.092
6.30	80	9.67 <sup>ab</sup>	0.377	1.091
Coefficient of	f variation (%)	1.41	2.19	0.12
Pv	alue	0.041	0.988	0.169

Averages followed by lowercase letters in the column differ from each other by the Tukey Test (5%).

Gomez & Angeles (2009) who did not see effect of the amino acid threonine on the percentage of yolk and albumen in diets of light layers. Sá et al. (2007) also did not find internal egg quality responses, except for the Haugh unit that presented a quadratic effect in relation to the threonine levels studied in their research, estimating the requirement of threonine for lightweight laying hens of 0.487% and ideal threonine:lysine ratio of 70%.

Figueiredo et al. (2012) reported that although threonine is an important and essential amino acid, the internal components of the egg are not affected, mainly because they are associated with the physiological measurements of laying hens.

Rocha (2010) evaluated different threonine:lysine ratios in lightweight laying hens of Hy Line - 36 during the peak of production and verified that the levels of digestible threonine in the diet did not influence the percentage of albumen and yolk, for the threonine:lysine ratio estimated at 78% for laying hens in the 24-40 week age range.

There was no influence (P < 0.05) of digestible threonine levels on the shell thickness and egg specific density (Table 4). For the percentage of shell, it was observed that the level of 5.50 g kg<sup>-1</sup> of threonine presented higher percentages when compared with the level of 6.30 g kg<sup>-1</sup>; however, no regression effect was observed for this result.

Azzam et al. (2011) worked with the supplementation of 0.47, 0.57, 0.67, and 0.77 and 0.87% digestible threonine for laying hens at 40 to 48 weeks of age and found no effect on the percentage of shell. Figueiredo et al. (2012) found that the percentage of shell increased linearly with the threonine level increase in the highest lysine level in the diet of 0.879%. These authors stated that this result may be associated with amino acid imbalances with decreased food intake that may have adversely affected the intake of nutrients, such as calcium, phosphorus, vitamins, minerals and others, which are considered essential for the formation of egg shell.

### Conclusion

According to performance and egg quality, the nutritional requirement of threonine for lightweight laying hens in the

period from 29 to 45 weeks is 0.550%, with the digestible threonine:lysine ratio of 69%.

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