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Digestible threonine to lysine ratio in diets for laying hens aged 24-40 weeks

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ABSTRACT - Two-hundred sixteen white laying hens were used to assess the ideal ratio of digestible threonine:lysine in diets for laying hens at 24 to 40 weeks of age. Birds were assigned to a randomized block design, with six treatments, six replicates per treatment and six birds per experimental unit. The cage was used as the blocking criterion. Experimental diets contained different digestible threonine:digestible lysine ratios (65, 70, 75, 80, 85 and 90%) with 142 g/kg of crude protein. Experimental diets were formulated to be isonitrogenous and isocaloric with different contents of L-glutamic acid. Feed intake (g/hen/d), egg production (%), egg weight (g), egg mass (g/hen/d), feed conversion ratio (kg/dozen and kg/kg egg), eggshell weight (g), albumen weight (g), yolk weight (g) and body weight gain (g) were assessed. The maximum egg production was observed at 78% digestible threonine:digestible lysine ratio, while the best values of feed conversion ratio (kg/dozen egg) and feed conversion ratio (kg/kg of egg) were observed at 77.6% and 75%, respectively. Feed intake, egg mass and egg contents (yolk, albumen and eggshell) were not affected by treatments. The estimated digestible threonine:digestible lysine ratio of Hy-Line W36 laying hens at 24 to 40 weeks of age is 78%, corresponding to 5.70 g/kg of dietary digestible threonine.

Key Words: amino acids, egg production, ideal protein, white laying hens

Introduction

The Brazilian egg production has increased in the past few years. According to UBABEF (2013), the Brazilian egg production in 2012 was approximately 31 billion units and per capita consumption of 161.53 units/year.

The increase in egg production has been possible due the studies of the daily requirement of indispensable amino acids for laying hens. This knowledge allows for maximizing egg production, decreasing environmental impacts by reduction of nitrogen excretion and consequently reducing production costs.

In this context, formulation of diets based on the concept of ideal protein is very important, as the nitrogen excretion is reduced when laying hens are fed a profile of amino acids closer to their nutritional requirements. However, formulation of diets based on the crude protein concept may lead to a situation of a lack or excess of certain amino acids. As a result, the feed intake and productive performance of laying hens may be compromised. For laying hens in production phase, the adequacy of dietary amino acids is very important due to their requirements of protein for egg production.

Threonine is third limiting amino acid in corn- and soybean meal-based diets for laying hens. Threonine is related to metabolic processes like acid uric formation and protein synthesis. It has been verified that threonine might function as a nutrient immunomodulator in maintaining the intestinal barrier function (Azzam et al., 2011).

Because laying hens have low protein synthesis requirement and the threonine requirement is a large component of maintenance, a minimum threonine:lysine ratio may need to be met in formulating diets for laying hens (Martínez-Amescua et al., 1999). Studies have demonstrated that reducing dietary level of crude protein and using L-threonine supplementation has no negative effect on quantitative laying production (Gheisar et al., 2011).

Therefore, this study was conducted to determine the ideal ratio of digestible threonine to lysine in diets for Hy-Line W36 laying hens at 24 to 40 weeks of age.

Material and Methods

Two hundred sixteen Hy-Line W-36 laying hens from 24 to 40 weeks of age were assigned to a randomized block design with six treatments, six replicates and six hens per experimental unit. The cage was used as the blocking criterion. Laying hens were handled from 20 weeks old until the beginning of the trial, at 24 weeks old, following recommendations of the Hy-Line W36 Commercial Management Guide and fed according to Rostagno et al. (2005).

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The laying hens were confined in compact-type wire cages (25 × 45 cm) in a bi-level system. Nipple drinkers were provided in each cage and hens had free access to water. Feeders were provided for each cage with a capacity of two hens per cage. Hens were fed the experimental diets from 24 to 40 weeks of age. Experimental diets were offered for 16 weeks, divided into four periods of four weeks.

Before the experimental diets were provided, laying hens were individually weighed and randomly allotted to pens so that all pens had approximately the same average body weight (1,328 g) and egg production. The control of egg production was carried out in the period between 20 and 24 weeks of age. At 24 weeks of age, the laying hens received the experimental diets. Hens were given free access to water and experimental diets.

The treatments consisted of six levels of digestible threonine (4.75, 5.11, 5.48, 5.84, 6.21 and 6.57 g/kg), resulting in different digestible threonine:digestible lysine ratios, as follows: 65, 70, 75, 80, 85 and 90%. A basal diet was formulated with 4.75 g/kg of digestible threonine (Table 1). Experimental diets were prepared from the basal diet by varying the levels of L-threonine, which was added to the basal diet in substitution of cornstarch and the nonessential amino acid L-glutamic acid.

All diets were isonitrogenous and had the same ratio of essential amino acids in relation to lysine, except threonine, and 100, 25, 85, 92, 102, 124, 31, 67 and 120% to digestible methionine+cystine; digestible tryptophan; digestible isoleucine; digestible valine, digestible arginine, digestible leucine, digestible histidine, digestible phenylalanine and digestible phenylalanine + tyrosine, respectively. The digestible methionine + cystine:digestible lysine ratio was used as recommended by Brumano et al. (2010) and the digestible lysine:digestible other-amino-acids ratio was used according to the recommendations of Rostagno et al. (2005).

At the onset of production, the exposure of the animals to light was gradually increased by increments of 15 min per week to reach a constant photoperiod of 16 hours of light and 8 hours of darkness (16L:8D). Laying hens received additional artificial light provided by incandescent lighting.

The lowest and highest temperatures recorded were 19.4 and 28.2 ºC, respectively. According to the Hy-Line W36 Commercial Management Guide (Hy-Line of Brazil, 2009-2011), the temperature inside the laying house should be 18 to 27 ºC. Therefore, it can be inferred that the hens were exposed to thermal stress during the experimental period.

The effects of dietary treatment on egg production (%), feed intake (g/hen/d), feed conversion ratio (kg/dozen and kg/kg of egg), egg weight (g), egg mass (g/hen/d), eggshell weight (g), albumen weight (g), yolk weight (g) and mortality were evaluated. Individual body weight (g) was recorded at the beginning and end of the experiment.

Analysis of variance was performed by using the statistical analysis system SAEG (Sistemas para Análises Estatísticas, version 9.1). The digestible threonine:digestible lysine ratio was determined by the polynomial regression. Statistical significance was considered at P<0.05.

Table 1 - Composition of basal diet

Ingredient	g/kg
Corn	651.97
Soybean meal	138.36
Corn gluten meal 60%	16.37
Sorghum	50.00
Dicalcium phosphate	15.73
Limestone	93.23
Salt	5.09
Vegetable oil	9.70
L-lysine HCl	2.75
DL-methionine	3.55
L-arginine	0.44
L-valine	1.37
L-isoleucine	1.60
L-tryptophan	0.82
Vitamin premix[1]	1.00
Mineral premix[2]	0.50
Potassium carbonate	2.23
Choline	0.20
Antioxidant[3]	0.10
L-glutamic acid	4.47
L-threonine	0.38
Cornstarch	0.13
Calculated composition (g/kg)	
Metabolizable energy (kcal/kg)	2900
Crude protein (g/kg)	142.00
Linoleic acid (g/kg)	12.10
Digestible lysine (g/kg)	7.30
Digestible methionine + cystine (g/kg)	7.67
Digestible methionine (g/kg)	4.02
Digestible tryptophan (g/kg)	2.04
Digestible threonine (g/kg)	4.75
Digestible valine (g/kg)	6.94
Digestible histidine (g/kg)	3.39
Digestible isoleucine (g/kg)	6.42
Digestible arginine (g/kg)	7.67
Digestible leucine (g/kg)	13.00
Digestible phenylalanine (g/kg)	6.21
Digestible phenylalanine + tyrosine	10.60
Calcium (g/kg)	40.20
Available phosphorus (g/kg)	3.75
Sodium (g/kg)	2.25
Chlorine (g/kg)	2.00
Potassium (g/kg)	5.80

[1] Content per kg premix: vitamin A - 12,000,000 IU; vitamin D3 - 3,600,000 IU; vitamin E - 3,500 IU; vitamin B1 - 2,500 mg; vitamin B2 - 8,000 mg; vitamin B6 - 3,000 mg; pantothenic acid - 12,000 mg; biotin - 200 mg; vitamin K - 3,000 mg; folacin - 3,500 mg; niacin - 40,000 mg; vitamin B - 1,220,000 mcg; Se - 130 mg.
[2] Content per kg premix: Mn - 160 g; Fe - 100 g; Zn - 100 g; Cu - 20 g; Co - 2 g; I - 2 g.
[3] BHT - Butylated Hydroxytoluene.

Results and Discussion

Digestible threonine:digestible lysine ratios had no effect on feed intake by laying hens (Table 2). This result is consistent with the idea that laying hens may be unable to compensate threonine deficiency by increasing feed intake described by Martínez-Amescua et al. (1999). In addition, Valério et al. (2000) found no differences in the feed intake of hens from 21 to 36 wk of age that were fed diets with different levels of digestible threonine and 142 g/kg of crude protein.

Digestible threonine intake increased linearly with increasing dietary threonine levels (P<0.01) (Table 2). Because feed intake did not differ among treatments, as digestible threonine increased, threonine intake increased linearly. This fact enables the observation of the effects of threonine on laying hens performance. Regression analysis for egg weight resulted in a linear response since the egg weight decreased as the digestible threonine content of the diet increased ($\hat{Y} = 61.08 - 8.88x$, $R^2 = 0.80$) (Table 2). The average egg weight observed in the present study was lower than those presented by the Guidelines for the breed (58.08 g).

In contrast, Martinez-Amescua et al. (1999) observed increase in egg weight when the dietary threonine (threonine:lysine ratio of 62%) increased from 4.3 g/kg to 4.7 g/kg (threonine:lysine ratio of 68%). Similarly, Faria et al. (2002) verified that egg weight increased as the threonine content of the diet increased. Additionally, Ishibashi et al. (1998), Valério et al. (2000) and Gomez & Angeles (2009) reported a lack of differences in egg weight due to increasing dietary levels of threonine. According to the last authors, these discrepancies may be due to the basal diets used in these studies.

Egg production increased quadratically, being highest at the 5.70 g/kg digestible threonine, corresponding to a digestible threonine:digestible lysine ratio of 78% ($\hat{Y} = -57.88 + 498.7x - 436.91x^2$, $R^2 = 0.50$) (Table 2).

The effect of threonine on egg production is well established and depends on the breed and age of the layer. Matos et al. (2009) reported that egg production is maximum at a digestible threonine:lysine ratio of 69% for Lohmann LSL laying hens at 24 to 44 weeks of age. Gomez & Angeles (2009) reported a threonine:lysine ratio of 65% for maximum egg production by Hy-Line W-36 laying hens at 100 weeks of age.

Feed conversion ratio (kg/dozen) showed a quadratic pattern (P<0.01), being greater with a dietary digestible threonine concentration of 5.67 g/kg, which was equivalent to a digestible threonine:digestible lysine ratio of 77.6% ($\hat{Y} = 3.481 - 7.648x + 6.74x^2$, $R^2 = 0.90$) (Table 3). This result corroborates the findings of Sá et al. (2007) and Matos et al. (2009), who found that dietary threonine influences the feed conversion ratio. Similarly, the quadratic regression of feed conversion ratio (kg/kg) indicated a requirement of 5.47 g/kg digestible threonine, corresponding to a digestible threonine:digestible lysine ratio of 75% ($\hat{Y} = 4.277 - 8.497x + 7.76x^2$, $R^2 = 0.81$). As such, the best digestible threonine: digestible lysine ratio to ensure optimal feed conversion ratio (77.6%) coincides with the best digestible threonine: digestible lysine ratio for maximum egg production (78%). Moreover, a digestible threonine:digestible lysine ratio higher than 75% resulted in decrease in egg weight. Egg mass was not affected by digestible threonine:digestible lysine ratio (Table 3). In contrast, Bregendahl et al. (2008) verified that the digestible threonine:digestible lysine ratio affected the egg mass of Hy-Line W-36 laying hens at 26 to 34 weeks of age, which was maximized at a threonine intake of 414 mg/hen/d, corresponding to 77% relative to lysine.

According to Sakomura & Rostagno (2007), 8.7 mg of digestible threonine is required for each gram of egg

Table 2 - Feed intake, digestible threonine intake, egg weight and egg production according to digestible threonine:digestible lysine ratio

Digestible threonine: digestible lysine ratio	Digestible threonine intake (g/kg)	Feed intake (g/hen/d)	Threonine intake (g/hen/d)	Egg weight (g/egg)	Egg production (%)
65	4.75	91.22	0.433	57.15	79.1
70	5.11	93.74	0.479	56.58	84.6
75	5.48	93.20	0.511	56.27	84.8
80	5.84	89.57	0.523	55.44	82.2
85	6.21	93.32	0.579	55.58	84.5
90	6.57	89.80	0.590	55.67	81.8
			P-value		
Linear effect		0.286	0.000	0.011	0.651
Quadratic effect		0.290	0.270	0.263	0.012
Lack of fit		0.123	0.112	0.657	0.127
CV (%)		3.51	3.53	2.12	3.70

produced. Considering that the average egg mass in the present study was 46.34 g/hen/d and that the lowest digestible threonine:digestible lysine ratio was sufficient to ensure this, 9.34 mg of digestible threonine was required for each gram of egg produced.

The average weight of yolk, albumen, eggshell and egg content in percentage in the hens receiving diets with 65 to 90% digestible threonine:digestible lysine ratio were not different (Tables 4 and 5). Therefore, it can be concluded that, for the levels of digestible threonine evaluated in this study, no major effects were observed on egg quality. Laying hens gained weight during the experimental period (Table 6). Koelkebeck et al. (1991) suggested that an excess of 1% of threonine seemed to have minimal effects on feed intake and weight gain in laying hens. Faria et al. (2002) verified a decrease in body weights of hens fed diets containing less than 4.5 g/kg threonine, which is lower than the levels evaluated in this study. The optimum ratios of digestible threonine:digestible lysine (Table 7) ranged from 75% to 90%. Considering that feed conversion ratio is an important production parameter, and an R^2 for regression equation of 0.90, the optimum digestible threonine:digestible

Table 3 - Feed conversion ratio and egg mass according to treatments digestible threonine:digestible lysine ratio

Digestible threonine: digestible lysine ratio	Digestible threonine (g/kg)	Feed conversion ratio (kg/dozen)	Feed conversion ratio (kg/kg egg)	Egg mass (g/hen/d)
65	4.75	1.37	2.00	45.59
70	5.11	1.32	1.94	48.21
75	5.48	1.31	1.93	48.06
80	5.84	1.32	1.98	45.22
85	6.21	1.34	2.00	46.58
90	6.57	1.35	2.01	44.42
			P-value	
Linear effect		0.876	0.176	0.076
Quadratic effect		0.006	0.058	0.360
Lack of fit		0.589	0.510	0.133
CV (%)		3.33	3.90	4.63

Table 4 - Yolk weight, albumen weight and eggshell weight according to digestible threonine:digestible lysine ratio

Digestible threonine: digestible lysine ratio	Digestible threonine (g/kg)	Yolk weight (g)	Albumen weight (g)	Eggshell weight (g)
65	4.75	14.79	37.04	5.00
70	5.11	14.80	37.30	4.78
75	5.48	14.89	37.18	4.88
80	5.84	14.72	36.26	4.90
85	6.21	14.63	36.81	4.86
90	6.57	14.66	36.96	4.99
			P-value	
Linear effect		0.258	0.477	0.805
Quadratic effect		0.628	0.621	0.217
Lack of fit		0.599	1.225	0.387
CV (%)		2.29	3.11	5.06

Table 5 - Yolk, albumen and eggshell contents according to digestible threonine:digestible lysine ratio

Digestible threonine: digestible lysine ratio	Digestible threonine (g/kg)	Yolk (g/100 g of egg)	Albumen (g/100 g of egg)	Eggshell (g/100 g of egg)
65	4.75	26.00	65.20	8.80
70	5.11	26.02	65.61	8.39
75	5.48	26.14	65.28	8.58
80	5.84	26.30	64.94	8.76
85	6.21	25.96	65.39	8.65
90	6.57	25.87	65.31	8.81
			P-value	
Linear effect		0.738	0.847	0.548
Quadratic effect		0.272	0.791	0.391
Lack of fit		0.629	0.344	0.399
CV (%)		2.19	1.09	5.61

Table 6 - Initial body weight, final body weight and change in body weight according to digestible threonine:digestible lysine ratio

Digestible threonine: digestible lysine ratio	Digestible threonine (g/kg)	Initial body weight (g)	Final body weight (g)	Body weight change (g)
65	4.75	1329.2	1438.9	109.7
70	5.11	1324.4	1444.3	119.9
75	5.48	1330.1	1443.2	113.1
80	5.84	1327.4	1433.9	106.5
85	6.21	1328.7	1465.4	136.7
90	6.57	1330.5	1416.5	86.0
			P-value	
Linear		0.407	0.768	0.710
Quadratic		0.489	0.517	0.483
Lack of fit		0.125	0.137	0.144
CV (%)		0.44	3.98	52.4

Table 7 - Digestible threonine:digestible lysine ratios according to the parameter evaluated

	Digestible threonine:digestible lysine ratio	Equation	R^2
Egg weight	>90%	$\hat{Y} = 61.08 – 8.88x$	0.80
Egg production	78%	$\hat{Y} = 57.88 + 498.7x – 436.91x^2$	0.50
Feed conversion ratio (kg/dozen)	77.6%	$\hat{Y} = 3.481 – 7.648x + 6.74x^2$	0.90
Feed conversion ratio (kg/kg of egg)	75%	$\hat{Y} = 4.277 – 8.497x + 7.76x^2$	0.81

lysine ratio of 77.6% for Hy-Line W-36 laying hens aged 24-40 weeks is recommended. In summary, the digestible threonine:digestible lysine ratio influences egg production, egg weight and feed conversion ratio, but does not affect the egg quality. The digestible threonine:digestible lysine ratio was estimated to be 77.6%. The digestible threonine: digestible lysine ratio determined in this study was higher than the 76% suggested by Rostagno et al. (2011).

Conclusions

For adequate productive performance of Hy-Line W36 laying hens at 24 to 40 weeks of age, the estimated digestible threonine:digestible lysine ratio is 77.6%.

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