



Digestible lysine levels for laying hens and their effects on egg quality

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ABSTRACT. Egg quality of semi-heavy laying hens fed on low protein diets (14.0% CP) and on different lysine levels is evaluated, while maintaining the same ratio of digestible amino acids / digestible lysine. Four hundred and twenty commercial strain Isa Brown laying hens, 28 weeks old, were divided into 42 experimental plots. A completely randomized design with six treatments and seven replicates was employed in four production cycles of 28 days each. Treatments comprised Control - 16.92% CP; 0.750% digestible lysine. Treatments 1 to 5, with CP levels 14% and digestible lysine levels 0.600, 0.675, 0.750, 0.825 and 0.900% respectively. Levels of Treatments 1 and 2 (0.546 and 0.640% digestible Met + Cys / 0.600 and 0.675% digestible lysine) provided smaller egg size. On the other hand, eggs had higher shell percentage when compared to control diet. When compared to other digestible amino acids, digestible lysine requirement may be estimated at 0.750% in a diet with 14% CP, which corresponds to the average daily intake of 876 mg dig. lysine hen⁻¹ day⁻¹ and 798 mg dig. Met + Cys hen⁻¹ day⁻¹, without jeopardizing performance and eggs' internal and external quality.

Keywords: ideal protein, digestible amino acids, Isa Brown, requirement, diet.

Níveis de lisina digestível para poedeiras semipesadas e seus efeitos sobre a qualidade dos ovos

RESUMO. A presente pesquisa objetivou avaliar a qualidade dos ovos de poedeiras semipesadas submetidas a dietas com baixa proteína (14,0% PB) e diferentes níveis de lisina, mantendo a mesma relação aminoácidos digestíveis/lisina digestível. Foram utilizadas 420 poedeiras da linhagem comercial Isa Brown, com 28 semanas de idade distribuídas em 42 parcelas experimentais. Foi utilizado um delineamento inteiramente casualizado com seis tratamentos e sete repetições, em quatro ciclos de produção de 28 dias/cada. Os tratamentos foram: Controle - 16,92% PB; 0,750% lisina digestível. Tratamentos 1 ao 5, com níveis de PB de 14% e lisina digestível de 0,600; 0,675; 0,750; 0,825 e 0,900%, respectivamente. Os níveis referentes aos tratamentos 1 e 2 (0,546 e 0,640% Met + Cys digestível / 0,600 e 0,675% de lisina digestível) proporcionaram menor tamanho do ovo; em contrapartida os ovos obtiveram maior porcentagem de casca, quando comparados à dieta controle. A exigência de lisina digestível em relação aos demais aminoácidos digestíveis, pode ser estimada em 0,750% em dieta com 14% de PB, o que corresponde ao consumo médio diário de 876 mg lisina dig. ave⁻¹ e 798 mg Met + Cys dig. ave⁻¹ dia⁻¹, sem comprometer o desempenho e qualidade interna e externa dos ovos.

Palavras-chave: proteína ideal, aminoácidos digestíveis, Isa Brown, exigência, dieta.

Introduction

Due to rise in production costs in animal nutrition caused by considerable increase in the prices of the main inputs, several studies have been undertaken on alternatives that might reduce crude protein levels for better economic results (KHAJALI et al., 2007) and lower environmental impact of the polluting excreta, mainly nitrogen (ROCHA et al., 2009) and ammonia emission rates (ROBERTS et al., 2007). Dudle-Cash (2012)

inferred that, in normal conditions, diets for breeding broilers contained up to 40% excess levels of digestible lysine. The author also highlighted the importance of defining optimal levels of digestible amino acids and thus reducing the diets' protein levels.

According to Sá et al. (2007), estimates of amino acid requirements for laying hens are affected by several factors that may impact individually or together. Although primary utilized for egg mass, a

lower percentage of daily necessities is used for maintenance. Since lysine plays an important metabolic function, the deficiency of this amino acid depresses growth and production, with consequent supplementation in diets for poultry. The above is due to the fact that cereals commonly used in feed composition contain low levels of amino acids (YAMANE et al., 2009).

Technological advances in large scale production of synthetic amino acids and accessible prices have facilitated their inclusion in the formulation of rations with lower crude protein rates (MOURA, 2004). According to Silva et al. (2000), the formulation of diets based on digestible amino acids is a new concept in nutrition in modern poultry production and actually represents a breakthrough. Jordão Filho et al. (2006b) report that increase in the performance of laying hens with supplementation of synthetic amino acids has been evaluated in many studies conducted in recent years and deserves attention with regard to the influence of amino acids on the internal quality of eggs in storage conditions. The internal and external quality of eggs has not received due attention in nutritional studies on laying hens when the effects of the specifications of amino acids in diets on the egg as human food is taken into account (JORDÃO FILHO et al., 2006b).

Genetic improvement has introduced new strains on the market and thus a considerable increase in productivity. In fact, Dudle-Cash, 2012, Figueiredo et al. (2012), Costa et al. (2008), and Jordão Filho et al. (2006a), point out that nutritional requirements should be evaluated and constantly renewed, together with the development of strains, since they are more efficient and productive. According to Jardim Filho et al. (2008), failure in nutrient supply results in poor quality eggs before impairment in the mobilization of nutrients for the maintenance of the reproductive system and the process of egg formation. This is particularly true when it comes to protein levels which determine production costs and depression of performance parameters and egg quality (ADEYEMO et al., 2012).

Current research evaluated the egg quality of semi-heavy laying hens fed on low protein diets (14.0% CP) and on different lysine levels, keeping the same digestible amino acids / digestible lysine ratio.

Material and methods

Current research was conducted at the Poultry Sector of the Department of Agricultural Sciences of Federal Institute of Minas Gerais, Bambuí Campus, Bambuí, Minas Gerais State, Brazil, during 16 experimental weeks, divided into four periods of 28 days each.

Four hundred and twenty commercial Isa Brown laying hens, initial age 28 weeks, were used, in a completely randomized design, divided into 6 treatments and seven replications. There were 42 experimental plots with 10 hens each, subdivided into 5 cages measuring 25 x 40 x 38 cm with 2 hens each. A selection of hens for egg production was conducted in the pre-trial period.

Treatments comprised Control, formulated according to nutritional requirements provided in the strain manual, with 16.92% crude protein (CP) and 0.750% digestible lysine. Treatment 1: 14.00% CP; 0.600% digestible lysine; Treatment 2: 14.00% CP; 0.675% digestible lysine; Treatment 3: 14.00% CP; 0.750% digestible lysine; Treatment 4: 14.00% CP; 0.825% digestible lysine; Treatment 5: 14.00% CP; 0.900% digestible lysine. Tables 1 and 2 show the nutritional composition and percentage of the experimental diets.

All diets of Treatments 1 through 5 were iso-nitrogenous (14% CP) with L-Alanine for nitrogen balance, isocaloric (2,800 kcal EM kg⁻¹), isocaloric (3.70%) and isophosphoric (0.35%) with different levels of lysine, keeping the same digestible amino acid / digestible lysine ratio (Table 3).

Hens were fed *ad libitum* within a light-dark program adopted at 16.5 hours of light / day provided by fluorescent light. Feeding, supplied in trough-type feeders, occurred twice a day and water was supplied from nipple-type drinkers.

Performance was evaluated for average feed intake (g hen⁻¹ day⁻¹). To calculate variables of internal and external quality of eggs, a representative sample of two whole eggs per experimental plot was collected, identified and weighed for subsequent analyses in the last two consecutive days of every 28-day period. Egg length and width (mm) were measured by a caliper (accuracy 0.02 mm) at their end and middle region. For specific gravity (g cm⁻³), all whole eggs produced per plot were analyzed for their specific gravity by immersion into 10 buckets containing saline solution at a density between 1.066 and 1.102 g cm⁻³, using an electronic oil densimeter for gauging.

Table 1. Composition of experimental diets and percentage of Isa Brown strain laying hens, aged 28 and 44 weeks, fed on respective Treatments.

¹ Composition and percentage of experimental diets						
Ingredients	Control	T1	T2	T3	T4	T5
Corn	62.432	68.467	68.181	67.895	67.609	67.323
Gluten Corn 60%	1.500	1.500	1.500	1.500	1.500	1.500
Far Soybeans 45%	23.735	12.129	12.164	12.199	12.234	12.269
Soy Oil	1.015	1.000	1.000	1.000	1.000	1.000
Limestone Powder	4.438	4.444	4.443	4.443	4.443	4.443
Limestone Gravel	4.438	4.444	4.443	4.443	4.443	4.443
Dicalcium Phosphate	1.417	1.506	1.507	1.508	1.509	1.510
^{1,1} Mineral Premix	0.100	0.100	0.100	0.100	0.100	0.100
^{1,2} Vitamin Premix	0.100	0.100	0.100	0.100	0.100	0.100
Salt	0.346	0.348	0.347	0.347	0.347	0.346
DL-methionine 99%	0.181	0.153	0.222	0.292	0.361	0.431
L-lysine HCL 99%	0.000	0.171	0.267	0.362	0.458	0.554
L-threonine 98.5%	0.000	0.052	0.111	0.169	0.227	0.285
L-tryptophan 98.5%	0.000	0.025	0.043	0.060	0.078	0.095
L-valine 98.5%	0.000	0.057	0.130	0.202	0.275	0.348
L-arginine 99%	0.000	0.000	0.060	0.120	0.179	0.239
L-isoleucine 99%	0.000	0.009	0.067	0.125	0.183	0.241
L-phenylalanine 99%	0.000	0.000	0.023	0.047	0.070	0.094
Alanine 99%	0.000	1.548	1.161	0.774	0.387	0.000
Kaolin	0.300	3.947	4.130	4.314	4.497	4.680
Total	100.00	100.00	100.00	100.00	100.00	100.00

¹Diets formulated following requirements proposed by the manual of strain ISA Hendrix Genetics Company, Nutrition Management Guide, (2007) and Rostagno, (2011); ^{1,1}Mineral Premix Composition per 100 g of product: manganese 7,5 mg, iron 5,000 mg, iodine 150 mg, zinc 7,000 mg, copper 850 mg, cobalt 20 mg; ^{1,2}Vitamin Premix Composition per 100 g product: vitamins: A 80,000 µ, B12 100 mg, D3 200,000 µ, E 1,500 mg, K3 200 mg, B2 400 mg, B6 100 mg, niacin 1,990 mg, pantothenic acid 535 mg, folic acid 20 mg, selenium 250 mg, antioxidant 10,000 mg.

Table 2. Chemical analyses of experimental diets, amino acid composition and nutritional percentage.

Nutritional Analysis (g 100 g ⁻¹ - Product in Natural Matter)						
*Support Analyses	Control	T1	T2	T3	T4	T5
Dry Matter	90.48	90.73	89.52	89.92	90.18	89.44
Crude protein	16.98	14.40	14.08	13.94	13.55	14.45
*Total Amino Acids						
Lysine	0.889	0.742	0.803	0.880	0.937	0.910
Threonine	0.649	0.490	0.598	0.622	0.641	0.663
Methionine	0.498	0.348	0.485	0.541	0.595	0.507
Cystine	0.247	0.213	0.230	0.204	0.207	0.222
Methionine + Cystine	0.745	0.562	0.716	0.745	0.802	0.730
Alanine	0.960	2.399	1.830	1.206	0.874	1.354
Arginine	1.182	0.773	0.768	0.789	0.844	0.834
Aspartic Acid	1.659	1.120	1.086	1.005	1.039	1.097
Glutamic Acid	3.358	2.297	2.273	2.026	2.221	2.408
Glycine	0.722	0.515	0.478	0.423	0.418	0.491
Histidine	0.425	0.336	0.344	0.302	0.333	0.305
Isoleucine	0.679	0.495	0.580	0.063	0.656	0.671
Leucine	1.609	1.294	1.293	1.170	1.226	1.331
Phenylalanine	0.893	0.668	0.672	0.660	0.750	0.705
Serine	0.880	0.617	0.611	0.558	0.575	0.638
Tyrosine	0.658	0.478	0.423	0.434	0.471	0.492
Valine	0.760	0.663	0.737	0.753	0.828	0.817
Tryptophan	0.190	0.148	0.169	0.190	0.190	0.191
* Free Amino Acids						
Lysine	0.012	0.148	0.271	0.309	0.406	0.317
Threonine	0.024	0.068	0.156	0.208	0.258	0.191
Methionine	0.215	0.169	0.291	0.348	0.421	0.301

*Analyses performed by Ajinomoto® do Brasil/ Ajinomoto Animal Nutrition.

Table 3. Digestible amino acids: digestible lysine ratio in experimental feeds of strain Isa Brown laying hens, aged between 28 and 44 weeks, fed on the respective treatments.

* Dig AA / Dig Lis	Control	T1	T2	T3	T4	T5
Arg / Lys	1.350	1.105	1.070	1.042	1.019	1.000
Ile / Lys	0.872	0.760	0.760	0.760	0.760	0.760
Met / Lys	0.582	0.585	0.622	0.651	0.675	0.695
M + C / Lys	0.910	0.910	0.910	0.910	0.910	0.910
Thr / Lys	0.760	0.760	0.760	0.760	0.760	0.760
Trp / Lys	0.233	0.230	0.230	0.230	0.230	0.230
Val / Lys	0.956	0.950	0.950	0.950	0.950	0.950
Leu / Lys	2.031	2.014	1.787	1.606	1.458	1.335
His / Lys	0.575	0.529	0.469	0.422	0.383	0.351
Phe / Lys	1.057	0.947	0.875	0.818	0.771	0.732
Phe + Tyr / Lys	1.799	1.620	1.473	1.356	1.260	1.180

*Amino acid ratios based on rates proposed by Rostagno (2011).

Further, the sampled eggs were broken for further assessment. Haugh unit: with the above-mentioned calipers, the height of dense albumen was recorded in the midline of the egg white by the equation:

$$UH = 100 \log. (H - G(30W^{-37} - 100)/100 + 1.9)$$

where:

H = height of albumen (mm);

G = 32.2;

W = egg weight (g), according to Haugh (1937).

Percentage of yolk and albumen: The yolk was separated and weighed on a precision balance (0.01 g). Calculations were made on a percentage based on the weight of the intact egg. Eggshell percentage: after breaking, the shells were washed and dried in an oven for 48 hours for subsequent weighing. Shell thickness (mm): thickness was obtained at three points in the middle region of the shell, with the aid of an analog micrometer (0.01 mm accuracy).

Data were statistically analyzed with statistical program, analysis of variance system SISVAR (FERREIRA, 2000), with contrasts by Scheffé test between Control and other Treatments. For Treatments 1 to 5 and experimental periods, the regression analysis was used for the quantitative levels of lysine.

Results and discussion

There was no significant effect ($p > 0.05$) of contrasts of each proposed Treatment when compared with the average of the Control for the variables: Haugh unit, percentage of yolk and albumen (Table 4).

It may be observed that, even with reduced protein at different levels of lysine, it is not enough to change the internal components of the eggs. The results are similar to those by Khajali et al. (2007), or rather, the maintenance of the digestible amino acid

/ digestible lysine ratio allowed the reduction of protein levels of the diets of laying hens without impairing the levels of internal egg quality. In their studies on laying hens aged between 34 and 50 weeks, Sá et al. (2007) failed to report any effect of the variation in digestible lysine levels on the Haugh unit parameter.

The above was also reported by Figueiredo et al. (2012) and Jardim Filho et al. (2008, 2010) who registered no individual effects of amino acids in the proportions of egg components (albumen and yolk). They noted that, although lysine and threonine were important essential amino acids, the internal components of the egg were not easily affected since they were associated with physiologic measurements of the laying hens (FIGUEIREDO et al., 2012).

There was no effect of the lysine level on the diets ($p > 0.05$) by regression analysis of Treatments 1 to 5, on the variables Haugh unit, yolk and albumen percentage (Table 5).

Results are similar to those by Rocha et al. (2009) for lysine levels between 0.545 and 0.770% for laying hens aged between 24 and 40 weeks. Similarly, Matos et al. (2009) found no effect of digestible lysine levels on variables assessed by regression.

A significant effect ($p < 0.05$) was reported on external egg quality, except for specific gravity (Table 6).

Significant effect on feed intake ($p < 0.05$) occurred only in Treatment 5 (0.900% dig. Lys.), with a low consumption when compared to Control (0.750% dig. Lys.). The above result differs from that reported by Silva et al. (2006) and suggests that reduction in the level of dietary CP from 16.5 to 15.25 and 14.00%, without amino acid supplementation, did not affect feed intake and conversion per mass and per dozen eggs ($p > 0.05$). It further suggests the possibility of using diets with higher protein levels below the recommended rate.

Table 4. Effect of different levels of lysine with the maintenance of the same ratios of digestible amino acids / digestible lysine on the variables Haugh unit, percentage of yolk and albumen.

Variables	Digestible lysine						CV
	Control	T1	T2	T3	T4	T5	
Haugh unit	88.28	89.14	90.57	89.33	89.58	88.64	4.66
Yolk %	24.35	24.96	24.47	24.63	24.71	24.04	5.5
Albumen %	65.56	64.71	65.20	65.26	65.05	65.86	2.17

Means followed by (** $p < 0.01$, * $p < 0.05$) in the line differ statistically from Control by Scheffé's test at 5% probability. CV = coefficient of variance; DMS = Minimum significant deviation.

Table 5. Effect of different lysine levels, maintaining the same ratios of digestible amino acids: digestible lysine for the experimental periods on the variables Haugh unit, percentage of yolk and albumen.

Variables	Digestible lysine levels %						Effect	R2
	0.600	0.675	0.750	0.825	0.900	CV		
Haugh	89.14	90.57	89.33	89.58	88.64	4.66	NS	0.56
Albumen %	64.71	65.20	65.26	65.05	65.86	2.17	NS	0.67
Yolk %	24.96	24.47	24.63	24.71	24.04	5.50	NS	0.57

NS - Not significant in regression ($p > 0.05$).

There were significant effects ($p < 0.01$) of Treatments on the length and width of the eggs. Treatments 1 and 2 provided lower length and width when compared to Control. Result is underscored by shell percentage since the smallest egg size produced the greatest shell deposition. The above is based on the influence of daily intakes of Met + Cys on the size and weight of the egg (BERTECHINI, 2006). A lower feed intake was observed in Treatments 1 and 2, respectively with 620 and 690 mg Met + Cys $\text{hen}^{-1} \text{day}^{-1}$, when compared to the consumption of 786 mg Met + Cys $\text{hen}^{-1} \text{day}^{-1}$ of Control.

In their studies on the effects of lysine and threonine levels in laying hens in a 42 to 58-week period, Figueiredo et al. (2012) reported a significant interaction between the amino acids and suggested an association between amino acid imbalance and decreased feed intake. In fact, it may have negatively affected the intake of nutrients (calcium, phosphorus, vitamins, minerals and others) essential for the formation of the eggshell. No significant effect on internal and external quality of eggs was reported by Lima et al. (2012) in a study of protein reduction with enzyme supplementation. The availability of similar nutrients in all diets, albeit with reformulation and / or reduced protein, may explain the fact.

There was a significant difference ($p < 0.05$) in shell thickness in Treatments 2 and 4 when compared to Control, with better thickness in Treatments 2 and 4. These results differ from those by Jardim Filho et al. (2008) in their studies on different digestive lysine levels with no significant effect on this particular variable. There was no

significant effect ($p > 0.05$) in specific gravity. Results were similar to those found by Jordão Filho et al. (2006a), Matos et al. (2009) and Figueiredo et al. (2012) who registered no effect of digestible lysine levels on this parameter.

When Treatments 1 to 5 were analyzed by regression, significant differences were observed ($p < 0.01$) for feed intake and egg width. However, the determination coefficient was low and the prediction equation could not be taken into consideration (Table 7).

Jardim Filho et al. (2010) registered a positive quadratic effect of supplemented digestible lysine for this parameter when studying 0.600, 0.700, 0.800 and 0.900% of digestible lysine for light laying hens, with lower feed intake at level 0.900%, respectively. Although there was a reduction of 2.92 percentage points of crude protein and digestible lysine levels ranging from 0.600 to 0.900%, it was not enough to affect its size and external component. Results agree with those by Jordão Filho et al. (2006a), who found no effects on eggshell quality eggs with lysine levels at peak production. This fact suggested that egg shell quality is not easily affected by dietary lysine supplementation.

Authors such as Jardim Filho et al. (2008, 2010) recommended a level of 600 mg kg^{-1} of lysine for two strains of light laying hens and considered level of 15.8% crude protein for good quality eggs for the 24-48-week period. The digestible lysine requirement for light laying hens aged 24 to 40 weeks, cited by Rocha et al. (2009), is at least a daily average consumption of 759 mg digestible lysine / hen or 14 mg lysine / egg gram.

Table 6. Effect of different lysine levels, with the same ratios of digestible amino acids: digestible lysine, on the variables feed intake (g), length and width (mm), shell thickness (mm), shell percentage and specific gravity (g cm^{-3}).

Variables	Treatments						CV	LSD
	Control	T1	T2	T3	T4	T5		
Feed Intake	115.15	113.65	112.54	116.82	112.69	110.46*	4.89	6.278
Length (mm)	57.73	56.98**	56.55**	57.77	57.66	57.08	2.15	1.1591
Width (mm)	44.44	43.62**	43.62**	44.17	44.16	44.10	1.91	0.7916
Thickness (mm)	0.535	0.541	0.549*	0.532	0.549*	0.536	4.26	0.0217
Shell perc. (%)	10.08	10.34*	10.33*	10.11	10.23	10.10	3.75	0.3596
Sp. Grav. (g cm^{-3})	1.0869	1.0913	1.0920	1.0907	1.0912	1.0909	0.57	---

Means followed by ** ($p < 0.01$) and * ($p < 0.05$) in the line differ statistically from Control by Scheffé's test at 5% probability. CV = Coefficient of variance; LSD = least significant deviation.

Table 7. Effect of different lysine levels at the same ratios of digestible amino acids: digestible lysine for Treatments 1 to 5 on the variables: feed intake ($\text{g hen}^{-1} \text{day}^{-1}$), egg length and width (mm), shell thickness (mm), shell percentage and specific gravity (g mL^{-1})

Variables	Digestible lysine percentage					CV	Effect	R ²
	0.600	0.625	0.750	0.825	0.900			
FI ($\text{g hen}^{-1} \text{day}^{-1}$)	113.65	111.73	116.82	112.69	110.46	5.10	Q*	0.43
Length (mm)	56.98	56.55	57.77	57.66	57.08	2.19	NS	0.36
Width (mm)	43.62	43.62	44.17	44.16	44.10	1.85	L**	0.69
Thickness (mm)	0.541	0.55	0.532	0.548	0.536	4.37	NS	0.07
Shell perc. (%)	10.34	10.33	10.11	10.23	10.10	3.76	NS	0.64
Spec. Grav. (g mL^{-1})	1.0913	1.0921	1.0906	1.0912	1.0909	0.15	NS	0.25

L** - linear effect ($p < 0.01$); NS - Non-significant effect ($p > 0.05$); CV - Coefficient of variance; R² - Coefficient of determination.

In the case of laying hens aged between 42 and 58 weeks, Figueiredo et al. (2012) concluded that, when aim was to maximize egg weight, egg mass and feed conversion, the dietary level recommended is 0.754, 0.772 and 0.804% digestible lysine, respectively. In the case of mass production and eggs of light laying hens, Matos et al. (2009) suggested levels 0.700 and 0.500% of digestible lysine and threonine, respectively, in 16% crude protein. For the level inferred in current research, or rather, 0.750% digestible lysine, threonine levels reached 0.760%, taking into account protein reduction from 16.92 to 14% crude protein.

In the case of semi-heavy laying hens, Sá et al. (2007) estimated requirements of digestible lysine, or rather, 0.732 and 0.715%, respectively, for 15% crude protein in light and semi-heavy laying hens, which corresponded to daily intakes of 893 and 804 mg of lysine for light and semi-heavy laying hens, respectively. At a higher level, Jordão Filho et al. (2006a) recommended 0.840% digestible lysine for laying hens aged between 30 and 46 weeks, and subjected to 17.1% crude protein.

Conclusion

The digestible lysine requirement is estimated at 0.750% on a diet with 14% CP, which corresponds to an average daily intake of 876 mg of excavation Lys hen⁻¹ day⁻¹, without affecting the internal and external quality eggs.

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