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Ideal digestible isoleucine:digestible lysine ratio in diets for laying hens aged 24-40 weeks

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ABSTRACT - Two hundred fifty-two Hy-Line W36 laying hens were allotted in a randomized block design with 6 treatments, 7 replicates and 6 hens per experimental unit in order to determine the ideal digestible isoleucine:digestible lysine (Ile:Lys) ratio for laying hens aged 24-40 weeks. Experimental diets contained 5.92, 6.33, 6.73, 7.14, 7.54 and 7.95 g/kg of digestible isoleucine, resulting in different Ile:Lys ratios (0.73:1, 0.78:1, 0.83:1, 0.88:1, 0.93:1 and 0.98:1). Experimental diets were isonitrogenous, with different dietary contents of glutamic acid, and made isocaloric by adjusting the levels of cornstarch. All essential amino acids were provided proportionally to digestible lysine. Feed intake, egg production, egg weight, egg mass, feed conversion ratio per egg mass, feed conversion ratio per dozen eggs, albumen, yolk and eggshell content were assessed. Feed intake, egg production, egg mass and feed conversion ratio were influenced by Ile:Lys ratios quadratically. The egg weight was not affected by Ile:Lys ratio. No differences were observed in the egg quality according to Ile:Lys ratios. The data indicates that the optimum digestible isoleucine:digestible lysine ratio for laying hens aged 24-40 week is 0.84:1, corresponding to the consumption of 681 mg of isoleucine and 811 mg of lysine/hen day^{-1}.

Key Words: digestible amino acid, egg mass, egg production, protein

Introduction

Isoleucine is an indispensable aminoacid for normal growth and development of laying hens as well as egg production and egg mass. It is one of the six deficient amino acids in corn-soybean meal diets (Shivazad et al., 2002). It is a branched-chain amino acid like valine and leucine.

The diets formulated based on the concept of ideal protein must have the exact balance of amino acids needed for optimum performance and maximum growth. Lysine is used as the reference amino acid to calculate the amount of the other amino acids in diets. The amount of amino acids to be added in the diet should not be expressed as percentage, but relative to lysine. Thus, requirements of amino acids would be calculated from the ideal ratios.

Methionine and lysine are amino acids routinely used in reduced-crude protein diets for laying hens. However, other synthetic amino acids are currently available in the market, such as tryptophan, threonine, isoleucine and valine. Besides the improvement of production, there are interests in the use of these amino acids as they are beneficial to the environment (i.e., nitrogen output from livestock and poultry are reduced) (Shivazad et al., 2002).

Therefore the balance of indispensable amino acids is important in diets for laying hens. It has been verified that the concentration of lysine in plasma can decrease with increase of dietary isoleucine concentration (Peganova & Eder, 2003). It is suggested that excess dietary isoleucine may affect fertility of broiler breeders (Ekmay et al., 2013) and that the digestible isoleucine:lysine ratio affects feed conversion and breast meat yield in broilers (Mejia et al., 2011). The studies on isoleucine requirement for broilers have been reviewed; however, there are limited recent studies assessing isoleucine requirements and isoleucine: lysine ratio for laying hens in the egg-laying phase.

It is supposed that the isoleucine requirement for laying hens has changed according to genetic changes and with the change of requirement of other essential amino acids, especially lysine. Therefore, the objective of this study was to evaluate optimum digestible isoleucine:digestible lysine ratios for laying hens at 24-40 weeks of age.

Material and Methods

Two hundred and fifty-two Hy-Line W-36 laying hens from 24 to 40 weeks of age were allotted in a randomized block design with 6 treatments, 7 replicates and 6 hens per experimental unit. The cage was considered a block.

The laying hens were confined in compact-type wire cages (25 × 45 cm) in a bi-level system. Nipple drinkers

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were provided in each cage and hens had free access to water. Trough feeders were provided for each cage to house 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 4 weeks.

The laying hens were handled from 17 weeks old until the beginning of the trial, following recommendations of the Hy-Line W36 Commercial Management Guide (Hy-Line of Brazil, 2009-2011), and they were fed according to Rostagno et al. (2005). In the period from 17 to 24 weeks of age, hens were fed a basal diet adjusted to meet their requirements as described by Rostagno et al. (2005). At the end of this period, they were individually weighed and allocated in the order of the same average live weight and egg production in each cage. At 24 weeks of age, hens started to receive the experimental diets.

Experimental diets contained different levels of digestible isoleucine, resulting in different digestible isoleucine:digestible lysine ratios, and were calculated for a feed intake of 90 g/hen day^{-1}. A basal diet with 5.92 g/kg of digestible isoleucine was formulated (Table 1). L-isoleucine was added to the basal diet at the expense of cornstarch and the nonessential amino acid L-glutamic acid, to provide 5.92, 6.33, 6.73, 7.14, 7.54 and 7.95 g/kg of digestible isoleucine, corresponding to 0.73:1, 0.78:1, 0.83:1, 0.88:1,

Table 1 - Composition of experimental diets

Ingredients (g/kg)	Treatments (Digestible isoleucine:Digestible lysine ratio)					
	0.73:1	0.78:1	0.83:1	0.88:1	0.93:1	0.98:1
Corn	623.52	623.52	623.52	623.52	623.52	623.52
Soybean meal	158.76	158.76	158.76	158.76	158.76	158.76
Limestone	103.47	103.47	103.47	103.47	103.47	103.47
Corn gluten 60%	50.00	50.00	50.00	50.00	50.00	50.00
Dicalcium phosphate	17.84	17.84	17.84	17.84	17.84	17.84
Salt	5.89	5.89	5.89	5.89	5.89	5.89
Soybean oil	22.09	22.09	22.09	22.09	22.09	22.09
Potassium carbonate	2.04	2.04	2.04	2.04	2.04	2.04
Dl-methionine	3.21	3.21	3.21	3.21	3.21	3.21
L-lysine HCl	2.96	2.96	2.96	2.96	2.96	2.96
L-threonine	1.23	1.23	1.23	1.23	1.23	1.23
L-valine	1.47	1.47	1.47	1.47	1.47	1.47
L-tryptophan	0.64	0.64	0.64	0.64	0.64	0.64
Vitamin premix[1]	1.00	1.00	1.00	1.00	1.00	1.00
Trace mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50
Choline	0.20	0.20	0.20	0.20	0.20	0.20
Antioxidant	0.10	0.10	0.10	0.10	0.10	0.10
L-isoleucine	0.50	0.92	1.33	1.74	2.19	4.21
L-glutamic acid	4.92	4.41	3.92	3.41	2.88	0.42
Cornstarch	0.04	0.13	0.22	0.31	0.40	0.83
Calculated composition (g/kg)[3]						
Linoleic acid	25.00	25.00	25.00	25.00	25.00	25.00
Calcium	44.7	44.7	44.7	44.7	44.7	44.7
Available phosphorus	4.17	4.17	4.17	4.17	4.17	4.17
Potassium	6.44	6.44	6.44	6.44	6.44	6.44
Sodium	2.50	2.50	2.50	2.50	2.50	2.50
Metabolizable energy (kcal/kg)	2905	2906	2907	2908	2909	2913
Crude protein	163.5	163.5	163.5	163.5	163.5	163.5
Digestible arginine	8.27	8.27	8.27	8.27	8.27	8.27
Digestible phenylalanine	7.57	7.57	7.57	7.57	7.57	7.57
Digestible phenylalanine+ tyrosine	13.16	13.16	13.16	13.16	13.16	13.16
Digestible histidine	3.84	3.84	3.84	3.84	3.84	3.84
Digestible isoleucine	5.92	6.33	6.73	7.14	7.54	7.95
Digestible lysine	8.11	8.11	8.11	8.11	8.11	8.11
Digestible leucine	16.27	16.27	16.27	16.27	16.27	16.27
Digestible methionine + cystine	8.11	8.11	8.11	8.11	8.11	8.11
Digestible methionine	5.74	5.74	5.74	5.74	5.74	5.74
Digestible threonine	6.33	6.33	6.33	6.33	6.33	6.33
Digestible tryptophan	2.03	2.03	2.03	2.03	2.03	2.03
Digestible valine	7.46	7.46	7.46	7.46	7.46	7.46
Isoleucine:lysine ratio	73	78	83	88	93	98

[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; folic acid - 3,500 mg; niacin - 40,000 mg; vitamin B12 - 20,000 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] Values proposed by Rostagno et al. (2005).

0.93:1 and 0.98:1 digestible isoleucine:digestible lysine ratios, respectively. All diets were isonitrogenous.

All experimental diets had the same ratio of essential amino acids in relation to lysine: digestible methionine + cystine, 100; digestible tryptophan, 25; digestible threonine, 78; digestible valine, 92; digestible arginine, 102; digestible leucine, 200; digestible histidine, 47; digestible phenylalanine, 93; and digestible phenylalanine + tyrosine, 162.

The digestible lysine level used was recommended by Rocha (2006). The digestible methionine + cystine: digestible lysine ratio used followed the recommendations of Brumano (2008), while digestible threonine:lysine ratio was according to the recommendations of Rocha et al. (2009), and digestible tryptophan:digestible lysine ratio was according to the recommendations of Calderano et al. (2010).

With exception of amino acids and crude protein, the other nutrients of the experimental diets were added at the levels recommended by Rostagno et al. (2005).

Fresh water was available *ad libitum*.

Laying hens received 17 h light and 7 h dark. Additional artificial light was provided by incandescent lighting.

The effects of dietary treatment on egg production, egg weight, egg mass, feed intake, feed conversion ratio and albumen, yolk and eggshell contents were assessed. Eggs were collected every day in order to determine egg production per experiment unit. Egg weight, egg mass, feed conversion ratio, albumen, yolk and eggshell contents were recorded and compiled every 28 days. Hens were individually weighed at the beginning and at the end of the experiment to determine the body weight gain.

Eggs were cracked and separated into yolk, shell and albumen fractions. Net weights were recorded separately for all 3 components from each egg. The shells with membranes were washed to remove the adhering albumen. The weights of shells and yolks were recorded and albumen weight was calculated as the difference between the weight of egg and weight of the yolk and eggshell. These results were presented as g content/100 g of egg.

Data were subjected to ANOVA using the statistical analysis system SAEG (Sistema para Análises Estatísticas e Genéticas, version 9.1). Significance of difference was based on the probability of a type I error set at $P<0.05$.

Results and Discussion

Feed intake, egg production, egg mass and feed conversion ratio were quadratically affected by digestible isoleucine:digestible lysine ratios ($P<0.05$). The egg weight was unaffected by digestible isoleucine:digestible lysine ratio ($P>0.05$; Table 2).

The feed intake was maximized at an isoleucine:lysine ratio of 0.86:1. The excess dietary isoleucine has been associated with a reduction in feed intake in laying hens (Peganova & Eder, 2002). The obvious assumption was that effects of excess dietary isoleucine might also be due to a secondary tryptophan deficiency in the brain (Peganova & Eder, 2002). Peganova et al. (2003) reported the existence of interaction between dietary neutral amino acids (isoleucine, valine, leucine, phenylalanine and tyrosine) and tryptophan in laying hens. According to the same authors, laying hens responded to low dietary tryptophan concentrations with markedly lower feed intake. In pigs it has been shown that high dietary neutral amino acids concentrations reduce feed intake due to a suppression of the transfer of tryptophan to the brain across the blood-brain barrier and subsequent formation of serotonin in the brain (Henry et al., 1992).

Egg mass increased quadratically with supplementation of isoleucine and the response was maximized at an isoleucine:lysine ratio of 0.84:1.

The reduction of egg production end egg mass with higher isoleucine:lysine ratios might be explained by lower feed intake at this ratios and consequently lower nutrient intakes, impairing the egg formation.

Table 2 - Performance means according to treatments

	Treatments (Digestible isoleucine:Digestible lysine ratio)						CV (%)	P-value		
	0.73:1	0.78:1	0.83:1	0.88:1	0.93:1	0.98:1		Linear	Quadratic	Lack of fit
FI (g/hen d^{-1})[1]	82.1	85.9	83.3	85.8	84.9	82.5	3.1	0.843	0.011	0.082
EP (%)[2]	81.03	84.01	84.11	85.04	83.63	76.39	4.5	0.055	0.000	0.456
EW (g/egg)	57.62	57.72	57.84	57.81	57.54	57.80	1.66	0.921	0.815	0.924
EM (g/hen d^{-1})[3]	46.06	48.45	46.62	48.57	47.55	44.10	4.07	0.097	0.000	0.048
FCR (kg/dz)[4]	1.25	1.23	1.22	1.25	1.23	1.32	4.4	0.062	0.010	0.315
FCR (kg/kg)[5]	1.82	1.77	1.82	1.81	1.79	1.91	4.1	0.054	0.030	0.177
BW change (g)	22	43	22	63	35	43				

CV - coefficient of variation.
[1] Feed intake ($Y = -266.9x^2 + 371.4x - 43.82$; $R^2 = 0.60$).
[2] Egg production ($Y = -620.1x^2 + 843.6x - 201.4$; $R^2 = 0.90$).
[3] Egg mass ($Y = -284.2x^2 + 387.00x - 83.43$; $R^2 = 0.70$).
[4] Feed conversion ratio (kg/dz) ($Y = 5.68x^2 - 7.65x + 3.79$; $R^2 = 0.75$).
[5] Feed conversion ratio (kg/kg) ($Y = 6.40x^2 - 8.55x + 4.64$; $R^2 = 0.65$).

The different digestible isoleucine:digestible lysine ratios resulted in quadratic effect on feed conversion ratio, either per dozen or per egg mass. The feed conversion ratio per dozen was minimal at a 0.83:1 isoleucine:lysine ratio. On the other hand, the feed conversion ratio by mass was minimal at a 0.82:1 isoleucine:lysine ratio.

Similarly to the present experiment, Shivazad et al. (2002), working with Hy-Line W36 hens, re-evaluated their isoleucine requirements from a corn-soybean meal diet and suggested daily isoleucine requirements of 449.8, 497.0, and 469.0 mg/day for egg production, egg weight and egg mass, respectively. These levels are lower than the levels found in present experiment, which demonstrates the importance of re-evaluated requirements of laying hens, since in 10 years these birds have undergone genetics changes.

Peganova & Eder (2002) verified that a dietary isoleucine concentration higher than 8 g/kg caused a reduction in body weight of hens and dietary isoleucine concentrations higher than 10 g/kg caused a reduction in the daily egg mass. This effect might be explained by the antagonism between isoleucine, leucine and valine in relation to absorption in the intestine.

The ratio between branched-chain amino acids is as important as the ratio of these amino acids to lysine. In the present experiment, the proportion of these amino acids was different among treatments. Further studies should be conducted regarding the ratio between these amino acids. It has been verified that excessive concentration of isoleucine in diets of laying hens leads to decrease in animal performance, which can be significantly alleviated by increasing the concentrations of the two other branched-chain amino acids leucine and valine (Peganova & Eder, 2003).

The increased body weight observed indicates appropriate growth and development of laying hens in this study.

No differences were observed for albumen, yolk and eggshell contents among the treatments (Table 3). The lowest isoleucine:lysine ratio (0.73:1), which corresponded to a consumption of 486 mg of isoleucine and 666 mg of lysine/day, can provide a satisfactory egg quality for laying hens. However, feed intake, feed conversion ratio, egg production and egg mass were increased with higher isoleucine:lysine ratios.

The data suggest that isoleucine does not affect egg quality, since neither egg weight nor egg contents (albumen, yolk, eggshell) were improved with supplemental isoleucine.

The optimum isoleucine:lysine ratios ranged from 0.73:1 to 0.86:1 (Table 4). In the case of branched-chain amino acids, in addition to the requirement, tolerance for an excessive supply of aminoacid must be considered in order to optimize the animal performance (Peganova & Eder, 2002).

Considering that egg mass is an important production parameter because it associates egg production (%) and egg weight (g), the optimum isoleucine:lysine ratio for laying hens aged 24-40 weeks of 0.84:1 is recommended. This ratio is lower than the 0.79:1 suggested by Bregendahl et al. (2008) for laying hens at 26 to 34 weeks of age. The requirements of isoleucine seem to be affected by the age, being lower in older animals.

The average crude protein intake in the experimental period (13.45 g) was lower than the 16.00 g recommended by the Hy-Line W36 Commercial Management Guide (Hy-Line International, 2009-2011). However, the egg mass was similar to that recommended by the guide, indicating that reduction of crude protein in diets and supplementation of amino acids provides satisfactory egg production.

Table 3 - Yolk content (YC), albumen content (AC), eggshell content (EC) and body weight (BW) change according to the treatments

	Treatments (Digestible isoleucine:Digestible lysine ratio)						CV (%)	P-value		
	0.73:1	0.78:1	0.83:1	0.88:1	0.93:1	0.98:1		Linear	Quadratic	Lack of fit
YC (g/100 g of egg)	24.48	24.87	24.58	24.17	24.49	24.38	1.95	0.182	0.965	0.116
AC (g/100 g of egg)	65.86	65.40	65.85	66.36	65.88	66.03	1.01	0.196	0.763	0.153
EC (g/100 g of egg)	9.66	9.72	9.57	9.47	9.63	9.59	4.67	0.608	0.622	0.855

CV - coefficient of variation.

Table 4 - Isoleucine:Lysine ratios according to the parameter evaluated

	Isoleucine:Lysine ratio	Equation	R^2
Feed intake	0.86:1	$Y = -266.9x^2 + 371.4x - 43.82$	0.60
Egg production	0.83:1	$Y = -620.1x^2 + 843.6x - 201.4$	0.90
Egg mass	0.84:1	$Y = -284.2x^2 + 387.00x - 83.43$	0.70
Feed conversion ratio (kg/dz)	0.83:1	$Y = 5.68x^2 - 7.65x + 3.79$	0.75
Feed conversion ratio (kg/kg)	0.82:1	$Y = 6.40x^2 - 8.55x + 4.64$	0.65
Egg quality	0.73:1		

Conclusions

The results indicate that the optimum digestible isoleucine:digestible lysine ratio for Hy-Line W36 laying hens at 24-40 weeks of age is 0.84:1, corresponding to an intake of 681 mg of isoleucine and 811 mg of lysine/hen day^{-1}.

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