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## Short Communication

# Determination of digestible isoleucine:lysine ratio in diets for laying hens aged 42-58 weeks

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**ABSTRACT** - Two hundred and fifty-two Hy-Line W36 laying hens were allotted in a completely randomized design with 6 treatments, 7 replicates and 6 hens per experimental unit in order to determine the ideal ratio of isoleucine (Ile) in relation to lysine (Lys) to laying hens aged 42-58 weeks. Experimental diets contained digestible Ile at different levels, 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). A basal diet was formulated to provide Isoleucine in levels below recommendations. This diet was supplemented with L-isoleucine to make up the 6 diets. Each diet was made isonitrogenous by varying the dietary contents of glutamic acid and isocaloric by adjusting the contents of cornstarch. All essential amino acids were provided proportionally to lysine. Egg production, egg weight, egg mass, feed conversion ratio, albumen, yolk and eggshell contents were recorded and compiled at every 28-day period. No differences were observed in the performance over a wide range of dietary isoleucine concentrations from 5.76 to 7.73 g/kg corresponding to 0.73:1 to 0.98:1 Ile:Lys ratios. The lowest Ile:Lys ratio (0.73:1) was sufficient to ensure satisfactory performance of birds, corresponding to the consumption of 534 mg of isoleucine and 731 mg of lysine/day.

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

## Introduction

Reduction of the dietary protein content has been a practice used in laying hen nutrition. However, a progressive reduction of the dietary protein content may lead to a situation where certain amino acids such as valine and isoleucine affect the animal performance (Peganova & Eder, 2002). As a result, supplementation of these amino acids as a free form is necessary in these cases.

Formulation of diets based on the ideal protein concept allows the reduction of dietary crude protein content due to synthetic amino acid supplementation. The *ideal protein* can be defined as one that provides 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 rest of the amino acids in diets.

Therefore, assuming a constant ideal amino acid profile from the absorbed ideal protein, it is possible to determine the requirements of all amino acids after the determination of an individual amino acid requirement (Boisen et al., 2000). According to Bregendahl et al. (2008), ideal amino acid profile employs the concept that, even if the absolute amino acid requirements change due to genetic or environmental factors, the ratio between them is slightly affected. Thus, after determination of the ideal amino acid profile, the requirement for a single amino acid (lysine) can be determined experimentally from what is commonly seen in field conditions. Consequently, requirements of the rest of amino acids would be calculated from the ideal ratios.

Amino acids such as lysine, threonine and methionine + cystine have been extensively studied. However, to trace the profile of the ideal protein, requirements of other amino acids such as isoleucine must be determined. Isoleucine is one of the limiting amino acids in a low-protein, corn-soybean diet for laying hens (Harms & Russell, 2000).

The lack of information regarding the requirement of isoleucine for laying hens motivated this study. The objective was to evaluate an optimum digestible isoleucine:lysine ratio to crude protein-reduced diets for laying hens.

## Material and Methods

The experiment was conducted at the Poultry Section of the Department of Animal Science in Universidade

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Federal de Viçosa - Brazil, in the period from June to October 2010.

Two hundred and fifty-two (252) Hy-line W-36 laying hens from 42 to 58 weeks of age were used. Animals were confined in compact-type wire cages (25 × 45 cm) in a bi-level system. Cages were equipped with nipple drinkers with free access to water, and trough feeders, as to place 2 hens in each cage. Hens were fed the experimental diets from 42 to 58 weeks of age. Experimental diets were offered for 16 weeks, divided into four periods of 4 weeks.

Within 38 to 42 weeks of age, animals were fed a basal diet adjusted to meet the requirements according to Rostagno et al. (2005). At the end of this period, the laying hens were individually weighed and grouped by their body weight and average egg production.

The experiment procedures followed recommendations of the Hy-Line W36 Commercial Management Guide (Hy-Line of Brazil, 2009-2011), and animals were fed according to Rostagno et al. (2005) until the beginning of the trial.

A basal diet was formulated with 5.76 g/kg of isoleucine and 150 g/kg of crude protein (Table 1). Six layer diets were prepared from the basal diet by varying the levels of L-isoleucine. Experimental diets contained different levels of digestible isoleucine, resulting in different isoleucine:lysine digestible ratios and were calculated for the consumption of 95 g/bird/day. All diets were isonitrogenic. L-isoleucine was added to the basal diet at the expense of cornstarch and the nonessential amino acid L-glutamic acid, to give 5.76, 6.15, 6.54, 6.94, 7.33 and 7.73 g/kg of digestible isoleucine, corresponding to the following isoleucine:lysine ratios: 0.73:1; 0.78:1; 0.83:1; 0.88:1; 0.93:1 and 0.98:1, respectively.

All experimental diets had the same ratio of essential amino acids in relation to lysine, which had been previously shown to support maximum performance. The level of lysine was used as recommended by Rocha et al. (2009a). The methionine + cystine:lysine ratio was used following the recommendations of Brumano et al. (2010), threonine:lysine ratio was used as reported by Rocha et al. (2009b) and tryptophan:lysine ratio as recommend by Calderano (unpublished data). The lysine:other amino acids ratio was 2% higher than values proposed by Rostagno et al (2005).

Laying hens received additional artificial light provided by incandescent lighting to adjust 17 h of light and 7 h of darkness. The temperature (minimum and maximum) and relative humidity in the laying house were recorded daily and were maintained at 13 and 27 °C and 73% of relative humidity.

The effects of dietary treatment on laying hen performance were determined. Eggs were collected every day in order to determine egg production per experimental unit. Egg weight, egg mass, feed conversion ratio, albumen, yolk and eggshell contents were recorded and compiled at every 28-day period. The hens were individually weighed at the beginning and end of the experiment, in order to determine the body weight gain.

All the eggs produced during the last three consecutive days of every 28-day period were collected to measure egg weight, albumen, yolk and eggshell contents. Eggs were weighed, cracked, and separated into yolk, shell, and albumen fractions. Wet weights were recorded separately

Table 1 - Composition of basal diet

| Feed ingredients | g/kg |
|---|---|
| Corn | 656.04 |
| Soybean meal | 163.03 |
| Limestone | 98.94 |
| Corn gluten 60% | 24.06 |
| Dicalcium phosphate | 17.20 |
| Salt | 5.19 |
| Vegetable oil | 18.24 |
| Potassium carbonate | 1.97 |
| Dl-methionine | 3.43 |
| L-lysine HCl | 2.75 |
| L-threonine | 1.49 |
| L-valine | 1.83 |
| L-tryptophan | 0.64 |
| Vitamin premix[1] | 1.00 |
| Trace mineral premix[2] | 0.50 |
| Choline | 0.20 |
| Antioxidant | 0.10 |
| L-isoleucine | 0.35 |
| Glutamic acid | 3.00 |
| Cornstarch | 0.05 |
| Calculated composition[3] (g/kg) | |
| Linoleic acid | 23.24 |
| Calcium | 42.3 |
| Available phosphorus | 3.95 |
| Chlorine | 3.45 |
| Potassium | 6.11 |
| Sodium | 2.37 |
| Metabolizable energy (kcal/kg) | 2900 |
| Crude protein | 150.0 |
| Arginine | 8.04 |
| Phenylalanine | 6.81 |
| Phenylalanine + tyrosine | 11.68 |
| Histidine | 3.68 |
| Isoleucine | 5.76 |
| Leucine | 14.08 |
| Lysine | 7.88 |
| Methionine + cystine | 7.88 |
| Methionine | 5.67 |
| Threonine | 6.16 |
| Tryptophan | 1.97 |
| Valine | 7.88 |
| Isoleucine/lysine ratio | 73 |

[1] Content per kg premix: vitamin A - 12,000,000 IU; vitamin $D_3$ - 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] Values proposed by Rostagno et al. (2005).

for all 3 components from each egg. The shells with membranes were washed in order to remove adhering albumen. Shells and yolks were weighed. Albumen weight was calculated as the difference between the weight of the egg and weight of the yolk and eggshell.

Eggs collected during the last three consecutive days of every 28-day period were saved for measurement of egg weight. All eggs per experimental unit were weighed. The average egg weight was calculated by dividing the total weight of eggs collected by the number of eggs collected.

Mortality was recorded throughout the study with egg production and feed intake rates adjusted accordingly.

The experiment was carried out under a completely randomized design with six dietary treatments consisting of different levels of L-isoleucine. Each experimental unit consisted of a group of 6 hens. Data were subjected to ANOVA using the Statistical Analysis System SAEG (Sistema para Análises Estatísticas, version 9.1). Cages served as the experimental unit. Significance of difference was based on the probability of type I error set at P<0.05.

## Results and Discussion

No differences were observed in the performance of hens over a wide range of dietary isoleucine concentrations from 5.76 to 7.73 g/kg corresponding to a 0.73:1 to 0.98:1 isoleucine:lysine ratio (P>0.05; Table 2). The isoleucine:lysine ratios evaluated in this experiment did not affect (P>0.05) albumen, yolk or eggshell contents. Therefore, it can be inferred that the lower isoleucine:lysine ratio (0.73:1), which corresponded to a consumption of 534 mg of isoleucine and 731 mg of lysine/day, can provide satisfactory performance of laying hens.

The data of the present study indicates that performance of laying hens fed a low isoleucine level was not different from those fed a normal isoleucine level diet (Table 2). Several studies have shown no adverse effect of animal performance by reducing dietary crude protein, when the diets were supplemented with crystalline amino acids (Blair et al., 1999; Novak et al., 2006). The results observed in the present study are in accordance with these findings. However, no improvement of the productive parameters with increasing supplementation of isoleucine was detected.

The isoleucine requirement for optimum performance of the animals found in the present study was different from those previously reported from other authors. The lower isoleucine:lysine ratio studied (0.73:1) was necessary to ensure 82% of egg production, egg weight of 63.17 g and egg mass of 51.65 g/hen/d. Previous studies estimated isoleucine:lysine ratio for laying hen within 0.79:1 to 0.83:1 (Coon & Zhang, 1999; Harms & Russel, 2000; Rostagno et al., 2005; Bregendahl et al., 2008). According to Boisen et al. (2000) the amino acid requirements of farm animals are influenced by different factors including population, weight, daily gain, gender and genotype environment and health status. However, the authors reported that most changes in amino acid requirements do not lead to changes in the relative proportion of the different amino acids.

In this context, the isoleucine requirement in absolute levels may vary according to egg production and egg mass. Nevertheless, by determining the isoleucine:lysine ratio, adjustments of absolute levels of dietary amino acids according to the level of production of layer hens can also be made, keeping the ratio of amino acids.

The lack of effects of increasing levels of dietary isoleucine may have occurred due to the inconstancy of isoleucine:branched-chain amino acids (BCAA) ratio. Thus, as the level of dietary isoleucine increased, the ratio of these amino acids decreased. As a consequence, an excess of isoleucine in relation to BCAA may have occurred due to the antagonism of this amino acid and two other BCAA (valine and leucine), leading to reduction of laying hen

Table 2 - Performance of hens according to dietary isoleucine:lysine ratios and body weight (BW) change according to treatments[1]

| | Treatments (isoleucine:lysine ratio) | | | | | | CV (%) | P value |
|---|---|---|---|---|---|---|---|---|
| | 0.73:1 | 0.78:1 | 0.83:1 | 0.88:1 | 0.93:1 | 0.98:1 | | |
| Feed intake (g/hen/d) | 93 | 94 | 93 | 92 | 93 | 93 | 1.9 | 0.622 |
| Egg production (%) | 82 | 82 | 83 | 81 | 82 | 81 | 4.0 | 0.679 |
| Egg weight (g) | 62.59 | 63.16 | 63.31 | 63.33 | 62.68 | 63.92 | 1.9 | 0.374 |
| Egg mass (g/hen/d) | 51.49 | 51.71 | 52.66 | 51.16 | 51.40 | 51.46 | 3.5 | 0.713 |
| Feed conversion (kg/dz) | 1.36 | 1.38 | 1.34 | 1.37 | 1.36 | 1.38 | 3.9 | 0.673 |
| Feed conversion (kg/kg) | 1.81 | 1.82 | 1.76 | 1.80 | 1.81 | 1.80 | 3.4 | 0.629 |
| Yolk content (g/100g of egg) | 25.95 | 26.12 | 25.90 | 26.06 | 26.02 | 25.71 | 2.5 | 0.886 |
| Albumen content (g/100g of egg) | 64.66 | 64.59 | 64.84 | 64.63 | 64.53 | 64.85 | 1.1 | 0.937 |
| Eggshell content (g/100g of egg) | 9.39 | 9.29 | 9.26 | 9.31 | 9.46 | 9.44 | 3.3 | 0.788 |
| Body weight change (g/16wk) | +61.1 | +85.9 | +120.5 | +77.7 | +80.5 | +63.8 | | |

[1] Not significant (P>0.05). CV - coefficient of variation.

performance. However, the ideal ratio between these amino acids has not been reported in previous studies.

Peganova & Eder (2003) reported that an excessive concentration of isoleucine in laying hen diets leads to decrease in animal performance, which can be significantly improved by increasing the concentrations of leucine and valine. However, in the current study, the increase in isoleucine levels was not accompanied by an increase of leucine and valine levels, which might have contributed to the genetic potential express of the hens.

According to Shivazad et al. (2002), reduction of amino acids such as isoleucine can be done by eliminating excess of other amino acids without challenges in the adequate amount of amino acids in the diet. These authors suggested a daily isoleucine requirement of 449.8, 497.0, and 469.0 mg/day for egg production, egg weight, and egg mass, respectively, to Hy-line W36 hens.

Therefore, the ratio between isoleucine:BCAA is as important as the ratio of these amino acids to lysine. However, for experimental purposes, it becomes difficult to isolate each factor without interference from the other.

Although the isoleucine:lysine ratio found in this study was lower than that recommended by the Hy-line W36 Commercial Management Guide (Hy-line of Brazil, 2009-2011), it was observed that average egg weight was greater than the weight reported in the strain guideline (63.92 × 62.0 g). The strain guideline suggests an intake of 561 mg of isoleucine, with 710 mg of lysine, which results in an approximate 0.79:1 isoleucine:lysine ratio. The values of feed conversion ratio (kg/dz, kg/kg) observed in the current study were similar to those reported in the strain guideline.

Through the experimental period, hens consumed less crude protein (13.95 g) than recommended by the Hy-line W36 Commercial Management Guide (Hy-line International, 2009-2011) (16.05 g), which may have contributed to no response by animals to isoleucine supplementation and the lower egg production (82%) compared with egg production reported by the strain guideline.

Silva et al. (2010) verified that 12% of crude protein in the diet (corresponding to an intake of 13.13 g of crude protein) supplemented with synthetic amino acids did not affect egg production. However, egg weight, egg mass and albumen percentage were influenced linearly by the increases of the crude protein level until 18% (corresponding to an intake of 20.64 g of crude protein). Therefore, it is important to supply the sufficient amount of dietary crude protein, allowing satisfactory performance of the animals and avoiding reduction in productivity due to limiting amino acids.

Rocha (unpublished data), in a study evaluating the influence of different isoleucine:lysine ratios on Hy-Line W36 hens aged 24 to 40 weeks, found a quadratic effect of treatments on egg production, egg weight and egg mass. This fact evidences that the requirement of laying hens changes as the animals age, so it is important to provide balanced diet to the different phases of egg production.

In summary, a 0.73:1 isoleucine:lysine ratio can be maintained, changing only the absolute amounts of amino acids adjusted for each level of egg production.

## Conclusions

The data indicate that the optimum isoleucine:lysine ratio for laying hens is 0.73:1, corresponding to the intake of 534 mg isoleucine and 731 mg lysine/day. Isoleucine may not be the fourth limiting amino acid and the isoleucine:branched chain amino acids ratio is as important as the isoleucine:lysine ratio.

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