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Digestible tryptophan-to-digestible lysine ratio in diets for laying hens of 42 to 58 weeks of age

Relação triptofano digestível:lisina digestível em dietas para poedeiras de 42 a 58 semanas de idade

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SUMMARY

To determine the ideal digestible tryptophan-to-digestible lysine ratio in diets for laying hens of 42 to 58 weeks of age, 240 Hy-Line W-36 hens at 42 weeks of age were distributed in a completely randomized design with five treatments, eight replicates and six birds per experimental unit. At 42 weeks of age, the birds were subjected to experimental treatments that consisted of diets with equal amounts of nutrients, except for the digestible tryptophan level. The digestible tryptophan levels in the experimental diets were 0.149, 0.160, 0.171, 0.182 and 0.193%, generating digestible tryptophan-to-digestible lysine ratios of 21.5, 23.1, 24.6, 26.2 and 27.8%. The digestible lysine level in the diets was sub-optimal (0.694%). The same ratios between lysine and the other amino acids were maintained in all experimental diets. The ideal digestible tryptophan-to-digestible lysine ratio was estimated through the studied parameters using analysis of variance and polynomial regression analysis (α = 0.05). The digestible tryptophan-to-digestible lysine ratio in diets quadratically affected egg production, egg mass, feed conversion per egg mass and use efficiency of digestible lysine for egg mass. However, there were no effect (P>0.05) on egg weight, feed conversion per dozen eggs, use efficiency of digestible lysine for number of eggs produced, percentage of egg components and weight gain. The digestible tryptophan-to-digestible lysine ratio estimated for theses parameters ranged from 23.6 to 24.3%. The digestible tryptophan-to-digestible lysine ratio recommended in diets for laying hens of 42 to 58 weeks of age is 24.3%.

Keywords: amino acid, bird, egg, ideal protein

RESUMO

Para determinar a relação ideal triptofano digestível:lisina digestível em dietas para poedeiras de 42 a 58 semanas de idade, 240 poedeiras Hy-Line W-36 com 42 semanas de idade foram distribuídas em delineamento inteiramente ao acaso com cinco tratamentos, oito repetições e seis aves por unidade experimental. Com 42 semanas de idade as aves foram submetidas aos tratamentos experimentais, que consistiram de dietas com quantidades iguais de nutrientes, exceto para o nível de triptofano digestível. Os níveis de triptofano digestível nas dietas experimentais foram 0,149; 0,160; 0,171; 0,182 e 0.193%, gerando relações triptofano digestível:lisina digestível de 21,5; 23,1; 24,6; 26,2 e 27,8%. O nível de lisina digestível nas dietas foi sub-ótimo (0,694%). As mesmas relações entre a lisina e outros aminoácidos foram mantidas em todas as dietas experimentais. A relação ideal triptofano digestpivel:lisina digestível foi estimada por meio dos parâmetros estudados utilizando análise de variância e análise de regressão polinomial (α = 0.05). A relação triptofano digestível:lisina digestível nas dietas influenciaram quadraticamente a produção de ovos, massa de ovos, conversão alimentar e a eficiência de utilização de lisina digestível para massa de ovos. No entanto, não houve efeito (P> 0,05) sobre o peso dos ovos, conversão alimentar por dúzia de ovos, eficiência de utilização de lisina digestível para número de ovos produzidos, porcentagem dos componentes dos ovos e ganho de peso. A relação ideal triptofano digestível:lisina digestível estimada para esses parâmetros variaram de 23,6 a 24,3%. A relação triptofano digestível:lisina digestível recomendada em dietas para poedeiras de 42 a 58 semanas de idade é 24,3%.

Palavras-chave: aminoácido, aves, ovos, proteína ideal

INTRODUCTION

Essential amino acids have an important role in the growth and production of laying hens; they are components of the body and egg proteins and important precursors of specialized products in the metabolism. Thus, the amino acid levels of diets should be precisely balanced.

Protein sources in laying hens diets have been partially replaced by industrial amino acids. This reduction is grounded in the ideal protein concept, which defines that essential amino acids should be expressed as ideal proportions relative to lysine. Lysine was chosen as the reference amino acid because it is used almost exclusively for protein deposition; it is a limiting amino acid in reduced-protein corn-soybean meal broiler diets; and its analysis is uncomplicated (BAKER, 1997). Once the ideal profile is established, the amino acids requirement of birds can be estimated from the lysine requirement. This is especially important because the absolute requirements of amino acids are largely influenced by genetic factors, production level and environmental factors, but the ratios between them are much less influenced (BREGENDAHL et al., 2008). Thus, research studies should be focused on establishment of ideal ratios between essential amino acids and lysine rather than determine the absolute requirement of the birds for each amino acid. However, the ideal profile of amino acids for commercial laying hens in the literature is not well-established.

Tryptophan is considered the third limiting amino acid in corn-soybean meal diets for laying hens (DEPONTI et al., 2007; HARMS & RUSSELL, 2000; PEGANOVA et al., 2003; RUSSELL & HARMS, 1999). It is the serotonin precursor and its deficiency causes birds to reduce their feed intake (PEGANOVA & EDER, 2003). Tryptophan is also important to alleviate the depression of weight gain and feed intake caused by niacin deficiency in poultry (Xie et al. 2014). However, few studies have been conducted with the objective to determine the requirement of digestible tryptophan and especially its ideal ratio with the digestible lysine for laying hens diets. Furthermore, the majority of studies on nutritional requirements of amino acids for laying hens are centered on the peak production, disregarding the post-peak production phase.

The objective of this study was to determine the ideal digestible tryptophan-to-digestible lysine ratio in diets for laying hens of 42 to 58 weeks of age.

MATERIALS AND METHODS

The experiment was conducted at the Poultry Section of the Department of Animal Science of The Federal University of Viçosa (UFV), located in Viçosa, Minas Gerais, Brazil. The municipality is located in a tropical climatic region (20° 45′ 45″S and 4° 52′ 04″W, with an altitude of 657m).

Two hundred and forty Hy-Line W-36 hens at 42 weeks of age, with initial average weight of 1,347 g, were housed in pairs in cages (25 × 40 × 45cm) installed in a poultry house (12 × 8 m) fenced with wire mesh and covered by a clay-tile gable roof. When the birds completed 40 weeks of age, they were weighed and distributed in a completely randomized design with five treatments with six replicates of eight birds per replicate. Egg production was recorded during the period of 40-42 weeks of age. Before the experimental diets were supplied, the birds were organized in the treatments according to egg production.

At 42 weeks of age, the birds were subjected to experimental treatments that consisted of diets with equal amounts of nutrients, except for the digestible tryptophan level. The digestible lysine level in the diets was sub-optimal (0.694%); this suboptimal level was important to ensure that all digestible lysine consumed was used, and so that the digestible tryptophan-to-digestible lysine ratio estimated reflected the real needs of the birds. The digestible tryptophan levels in the experimental diets were 0.149, 0.160, 0.171, 0.182, and 0.193%, providing digestible tryptophan-to-digestible lysine ratios of 21.5, 23.1, 24.6, 26.2, and 27.8%, respectively. The animals were supplemented with L-tryptophan in substitution of L-glutamic acid in protein equivalent. After this substitution, the inert (starch) was added in the amount necessary to balance the diets. The same ratios between lysine and the other amino acids were maintained in all diets (Table 1 and 2).

The ratios between the essential amino acids and digestible lysine in the diets were: methionine + cystine, 104%; threonine, 81%; methionine, 53%; valine, 93%; and isoleucine, 86%. The other nutrients and energy, except crude protein, followed the recommendations of Rostagno et al. (2005).

The birds were supplied with feed and water *ad libitum* and the lighting program adopted consisted of providing 17 hours of light per day.

The average maximum and minimum temperatures recorded inside the poultry house during the experiment were 26.5 and 14.8°C. The experiment lasted 16 weeks. The parameters measured were: feed intake, digestible tryptophan intake, digestible lysine intake, egg production, egg weight, egg mass, feed conversion per egg mass (kg/kg) and per dozen eggs (kg/dz), use efficiency of digestible lysine for eggs mass (g/g) and for number of eggs produced (n eggs/g), percentage of egg components and weight gain.

Table 1. Composition and nutritional value of the basal diet in natural matter

Composition	Basal
Corn (7.8%)	61.987
Soybean meal (44.3%)	19.373
Corn Gluten Meal (60%)	1.185
Soybean oil	4.546
Limestone	9.780
Dicalcium Phosphate	1.669
Salt	0.556
Potassium carbonate	0.151
Choline chloride (60%)	0.040
Antioxidant[a]	0.010
Vitamin mixture[b]	0.100
Mineral mixture[c]	0.050
L-Lysine HCl (79%)	0.075
DL-Methionine (99%)	0.280
L-Threonine (98%)	0.070
L-Isoleucine (98.5%)	0.036
L-Valine (98.5%)	0.023
L-Tryptophan (98%)	-
L-Glutamic Acid	0.069
Starch	-
Nutritional Composition (g/kg)	
Crude Protein (%)	14.5
Metabolizable Energy (kcal/kg)	3,000
Calcium (%)	4.230
Available Phosphorus (%)	0.395
Potassium (%)	0.615
Chloride (%)	0.373
Sodium (%)	0.237
Digestible Tryptophan (%)	0.149
Digestible Lysine (%)	0.694
Digestible Met+Cys (%)	0.722
Digestible Methionine (%)	0.503
Digestible Threonine (%)	0.562
Digestible Isoleucine (%)	0.597
Digestible Valine (%)	0.645

[a]Butylated hydroxytoluene; [b]Quantity per kg of feed: vit. A – 7,200 UI; vit D_3 – 1,600 UI; vit. E - 5 UI; vit B_1 – 0.9mg; vit B_2 – 2.7mg; vit B_6 – 1.5mg; calcium pantothenate – 5.9mg; biotin - 0.02mg; vit. K_3 – 1.1mg; folic acid – 0.25mg; niacin - 16mg; vit. B_{12} – 7.2mcg; selenium – 0.25mg; [c]Quantity per kg of feed: manganese - 75mg; iron - 50mg; zinc - 70mg; copper - 8mg; iodine – 0.75mg.

Table 2. Addition of L-Tryptophan, L-Glutamic acid and starch added in each treatment

Digestible tryptophan level	0.149	0.160	0.171	0.182	0.193
L-Tryptophan (98%)	-	0.011	0.023	0.034	0.045
L-Glutamic Acid	0.069	0.053	0.034	0.018	-
Starch	-	0.005	0.012	0.017	0.024

The ideal digestible tryptophan-to-digestible lysine ratio was estimated through the parameters studied using analysis of variance and polynomial regression analysis (the sum square of treatment was decomposed in sum square of tested regression model). We opted to test linear and quadratic regression model given the biological interpretation of the parameter estimates from these models. The models were compared by F-test and R^2. A confidence interval of 95% was calculated for the parameters with significant quadratic effect. The probability value adopted was $\alpha = 0.05$. Analyses were performed on statistical analysis system SAEG version 9.1 (2007).

RESULTS AND DISCUSSION

The digestible tryptophan intake of the birds increased ($P<0.05$) as the digestible tryptophan-to-digestible lysine ratio in the diets was elevated. Hens consumed between 133.8 and 168.9mg/day of digestible tryptophan. However, the tryptophan-to-digestible lysine ratio had no influence ($P>0.05$) on feed intake or digestible lysine intake (Table 3). The birds fed the lowest digestible tryptophan-to-digestible lysine ratio ingested 133mg/day of digestible tryptophan. Despite the known role of the tryptophan as a precursor of serotonin - the neurotransmitter to which the function of regulating appetite in birds has been attributed - the observed tryptophan intake was not low enough to suppress the feed intake or the birds ingest more feed in an attempt to achieve the required consumption of tryptophan that was deficient in the diet. Thus, the increase in the digestible tryptophan intake observed was largely due to the increase in the digestible tryptophan content in the diets. Deponti et al. (2007) came to the same conclusion and observed that the intake of 137mg/bird/day was not low enough to influence the feed intake of laying hens.

Egg production, egg mass, feed conversion per egg mass and use efficiency of digestible lysine for eggs mass were quadratically affected ($P<0.05$) by the digestible tryptophan-to-digestible lysine ratios (Figure 1 - 4). For the feed conversion per egg mass and use efficiency of digestible lysine for eggs mass there was a linear effect of the digestible tryptophan-to-digestible lysine ratios, however the quadratic model showed the best fit of the data with the highest R^2.

When the digestible tryptophan-to-digestible lysine ratios in the diets increased from 21.5 to 24.6%, egg production increased by 3.93%. For this parameter, the ideal level of digestible tryptophan estimated was 0.172%, according to the equation: $\hat{y} = -65.299 + 1708.5x - 4970.6x^2$ ($R^2 = 0.17$). This level corresponded to the intake of 153 mg/bird/day of digestible tryptophan and a digestible tryptophan-to-digestible lysine ratio of 24.8%. Applying the confidence interval of 95% to the quadratic response equation we obtained the ratio of 23.6%.

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Table 3. Performance of laying hens from 42 to 58 weeks of age fed with diets containing different ratios digestible tryptophan:digestible lysine

Parameter	Digestible tryptophan-to-digestible lysine ratio (%)					CV (%)	P Value	
	21.5	23.1	24.6	26.2	27.8		L[a]	Q[b]
Feed intake (g/bird/day)	89.78	88.48	90.34	87.69	87.50	3.16	0.097	0.542
Digestible tryptophan intake (mg/bird/day)	133.8	141.6	154.5	159.6	168.9	3.26	0.000	0.465
Digestible lysine intake (mg/bird/day)	623.1	614.0	626.9	608.6	607.3	3.16	0.097	0.542
Egg production (%)	79.11	80.18	82.22	80.67	79.33	3.67	0.776	0.038
Egg weight (g)	59.82	59.64	60.72	60.10	60.12	2.37	0.508	0.499
Egg mass (g/bird/day)	47.30	47.81	49.92	48.50	47.69	4.01	0.503	0.022
Feed conversion per egg mass (g/g)	1.899	1.852	1.811	1.810	1.835	2.90	0.007	0.013
Feed conversion per dozen eggs (kg/dozen)	1.363	1.323	1.319	1.305	1.324	3.44	0.065	0.078
Use efficiency of digestible lysine for eggs mass (g/g)	75.952	77.860	79.651	79.657	78.552	2.89	0.009	0.013
Use efficiency of digestible lysine for number of eggs (nº eggs/g)	1.271	1.308	1.313	1.327	1.307	3.39	0.078	0.081
Percentage of yolk (%)	26.25	26.23	26.27	25.91	26.19	3.19	0.635	0.851
Percentage of albumen (%)	64.38	64.45	64.24	64.81	64.42	1.28	0.636	0.894
Percentage of shell (%)	9.37	9.31	9.49	9.29	9.39	3.36	1.000	0.883
Weight gain (g/bird)	66.14	44.80	69.44	60.58	36.90	84.92	0.424	0.545

[a]Linear effect; [b]Quadratic effect.

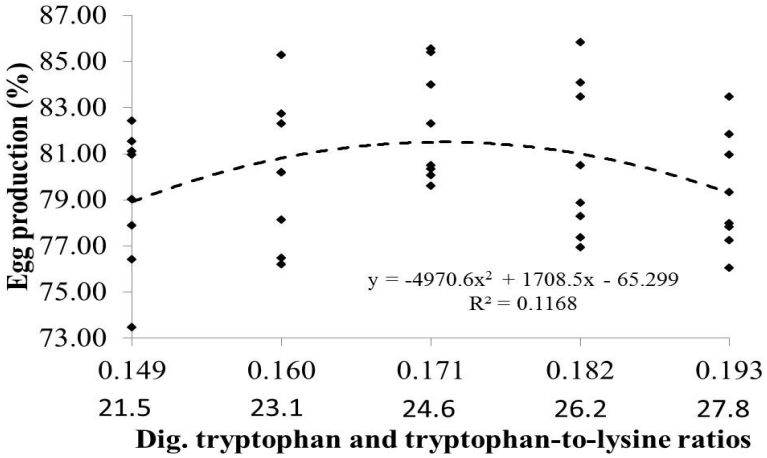

Figure 1. Effect of digestible tryptophan-to-digestible lysine ratio in the diet on egg production

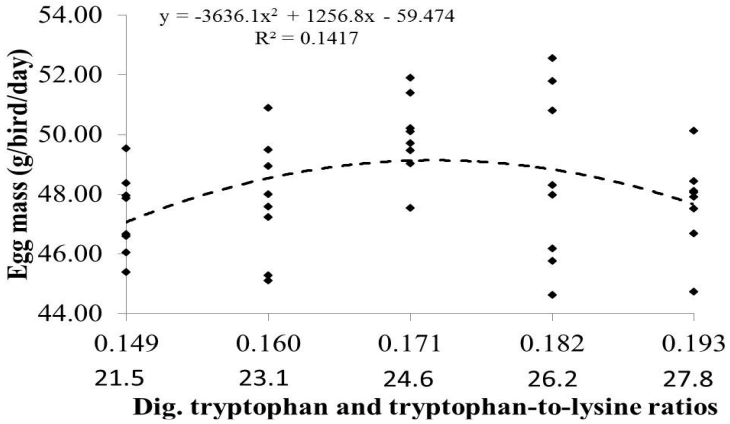

Figure 2. Effect of digestible tryptophan-to-digestible lysine ratio in the diet on egg mass

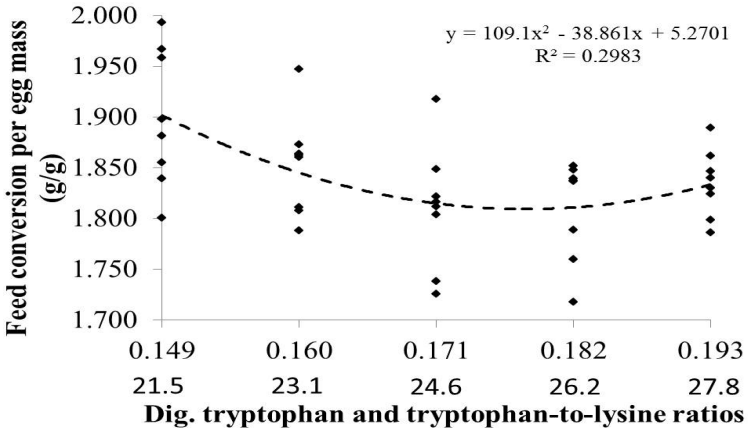

Figure 3. Effect of digestible tryptophan-to-digestible lysine ratio in the diet on feed conversion per eggs mass

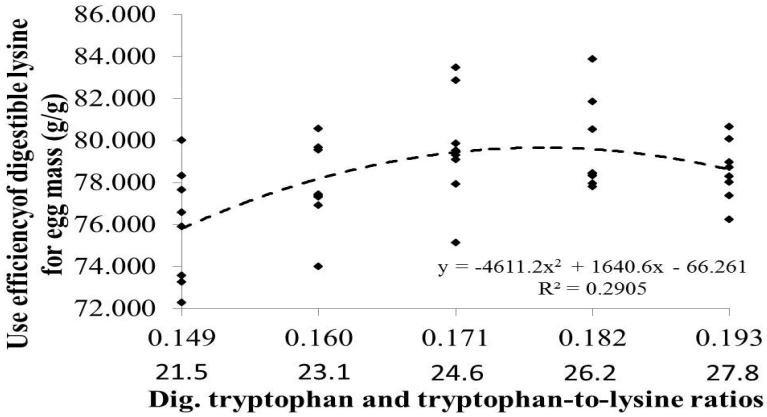

Figure 4. Effect of digestible tryptophan-to-digestible lysine ratio in the diet on use efficiency of digestible lysine for egg mass

The ideal digestible tryptophan-to-digestible lysine ratio of 23.6% estimated in this study for egg production was close to the 24.0% estimated for the same parameter by Lima et al. (2012), who studied the tryptophan-to-digestible ratios of 19, 21, 23, 25 and 27% in diets. The overall average egg production in the present study was 80.3%, which is below the expected according to the strain manual. This fact can be explained by the suboptimal level of digestible lysine used in the diets. Similarly, Brumano et al. (2010) and Reis et al. (2011), to estimate ideal ratios between essential amino acids and lysine for birds, also used suboptimal levels of digestible lysine in the diet. The suboptimal level is important to ensure that all digestible lysine consumed by birds are actually used and the digestible tryptophan-to-digestible lysine ratio is estimated accurately. A higher amount of dietary digestible lysine than what birds can use may lead to an underestimated digestible tryptophan-to-digestible lysine ratio. Because the objective with the study was to determine the ideal digestible tryptophan-to-digestible lysine ratio and not the absolute requirement of digestible tryptophan as mg/bird/day, this lower egg production did not influence the reliability of estimates.

Egg mass increased from 47.30 to 49.92g/bird/day when the digestible tryptophan-to-digestible lysine ratio in the diets was increased from 21.5 to 24.6%. The level of digestible tryptophan that maximized egg mass was 0.173%, which corresponded to the intake of 154 mg/bird/day of digestible tryptophan and a digestible tryptophan-to-digestible lysine ratio of 24.9%. 95% of this response was 23.7%. The equation for egg mass was: $\hat{y} = -59.474 + 1256.8x - 3636.1x^2$ ($R^2 = 0.14$).

The effect of digestible tryptophan-to-digestible lysine ratios observed on egg mass reflected the effect found in egg production. This finding was similar to that observed by other authors, who also noted the influence of tryptophan levels in the diet on the egg mass (PEGANOVA et al. 2003; RUSSELL & HARMS 1999). However, Russell & Harms (1999) observed that only levels of tryptophan lower than or equal to 0.130% promoted reduction in the egg mass produced by laying hens of 55 to 59 weeks of age. The ideal ratio of 24.3% estimated for feed conversion per egg mass, found in the present study, was similar to the 24.6%

obtained by Lima et al. (2012) for the same parameter.

Feed conversion per egg mass improved by 4.67% when the digestible tryptophan-to-digestible lysine ratios in the diets were increased from 21.5 to 26.2%. The optimal digestible tryptophan level estimated was 0.178%, according to the equation: $\hat{y} = 5.2701 – 38.861x + 109.1x^2$ ($R^2 = 0.30$). This level corresponded to the intake of 158 mg/bird/day of digestible tryptophan and to a digestible tryptophan-to-digestible lysine ratio of 25.6%. The confidence limit of 95% to the quadratic response equation resulted in the ratio of 24.3%.

Increasing the digestible tryptophan-to-digestible lysine ratio from 21.5 to 26.2% caused the egg mass produced per each gram of digestible lysine consumed to increase from 75.952 to 79.657 g. The equation estimated for the use efficiency of digestible lysine for egg mass was: $\hat{y} = –66.261 + 1640.6x – 4611.2x^2$ ($R^2 = 0.29$). The level of digestible tryptophan that maximized this parameter was 0.178%. This level corresponded to the intake of 158 mg/bird/day of digestible tryptophan and a digestible tryptophan-to-digestible lysine ratio of 25.6%. Applying the confidence interval of 95% to the quadratic response equation, we obtained the ratio of 24.3%.

As occurred in this present study, in experiments aimed to establish ideal ratios between essential amino acids and lysine, it is ideal that the feed intake of the birds for different treatments not present significant variation. A variation in feed intake results in variation in the amount of digestible lysine consumed, which could influence the performance parameters and mask the estimated ideal ratio. In these cases, the use efficiency of digestible lysine for egg mass can be an important parameter to help isolate the effect of digestible tryptophan on bird performance. Thus, the level of digestible tryptophan in the diet which maximizes egg mass and/or the number of eggs per gram of digestible lysine consumed is the one that establishes the best-fit or ideal digestible tryptophan-to-digestible lysine ratio. In the present study, the ideal digestible tryptophan-to-digestible lysine ratio for this parameter was 24.3%.

The digestible tryptophan-to-digestible lysine ratio had no significant effect ($P>0.05$) on egg weight, feed conversion per dozen eggs, use efficiency of digestible lysine for number of eggs produced, percentage of egg components and weight gain. Thus, the intake of 134mg/bird/day of digestible tryptophan or the digestible tryptophan-to-digestible lysine ratio of 21.5% was sufficient to maintain theses parameters. Similarly to the present study, Deponti et al. (2007) concluded that the egg weight, percentage of yolk and albumen and the total solids content of yolk and albumen of the eggs from laying hens are not influenced by ingestion of tryptophan in levels of 137.1 to 228.0mg/bird/day. Antar et al. (2004) also concluded that egg weight was not affected when the hens diets contained 0.166 or 0.176% of tryptophan.

The estimated digestible tryptophan-to-digestible lysine ratio, considering the studied parameters, ranged from 23.6 to 24.3%. Taking the feed conversion per egg mass into account, the estimated ideal digestible tryptophan-to-digestible lysine ratio was 24.3%. This value was similar to the 24.6% estimated by Lima et al. (2012) based on the feed conversion per egg mass of hens of 29 to 45 weeks of age. This ratio was higher than the values obtained by Deponti et al. (2007), who concluded that depending on the trait (production or egg mass) and on the regression model applied on data analyses (quadratic, exponential or segmented), the ideal tryptophan-to-lysine ratios for laying hens of 53 to 58 weeks of age are in the range of 17.4 to

20.3%. However, the authors adopted the methionine + cystine-to-lysine ratio of 81%. In a recent research study with laying hens, Brumano et al. (2010) estimated an ideal digestible methionine + cystine-to-digestible lysine ratio of 101% for laying hens of 42 to 58 weeks of age. It should be noted that even though the birds still have the potential to respond to increase in the digestible tryptophan-to-digestible lysine ratio in the diet, the performance gain may be limited if the ratios between other essential amino acids and digestible lysine are underestimated, and this may lead to underestimation of the digestible tryptophan-to-digestible lysine ratio. The digestible tryptophan-to-digestible lysine ratio estimated in this study was also higher than the 22.3% estimated by Bregendahl et al. (2008) for optimal egg mass by hens of 28 to 34 weeks of age. However, these authors used increasing levels of digestible lysine in the experimental diets, which can also make the estimate of the digestible tryptophan-to-digestible lysine ratio subjective.

The ideal digestible tryptophan-to-digestible lysine ratio recommended in diets for laying hens of 42 to 58 weeks of age is 24.3%.

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