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Egg Quality of Hens Fed Different Digestible Lysine and Arginine Levels

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ABSTRACT

This experiment aimed at evaluating the influence of the supplementation of digestible lysine and digestible arginine at different ratios in the diet fed to layers between 24 to 44 weeks of age on egg quality. In total,320 Lohmann LSL laying hens were allotted according to a completely randomized design in a 2 x 4factorial arrangement, consisting of two digestible lysine levels (700 or 900 mg/kg of diet) and four digestible arginine levels (700, 800, 900,or 1000 mg/kg of diet). Diets contained, therefore, digestible Lys:Arg ratios of 100, 114, 128, and 142 when the diet contained 700 mg digestible lysine per kg of diet, and 78, 89, 100, and 111 when 900 mg digestible lysine per kg was supplemented. The data obtained with digestible arginine levels were fitted to polynomial regression equations, and with digestible lysine, the F test (5% probability) was used to compare the means. The following variables were evaluated: egg weight; internal egg quality (yolk percentage and index, albumen percentage, Haugh units), eggshell quality (specific gravity andeggshell percentage); and whole egg, albumen, and yolk solids content. Digestible lysine and arginine interaction did not affect egg quality. Increasing levels of digestible lysine and arginine reduced eggshell quality and albumen solids, respectively. The levels of these amino acids suggested to improveegg quality are 700 mg digestible lysine and 700 mg digestible arginine/kg of feed at a Dig Lys: Dig Arg ratio of 100.

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INTRODUCTION

According to Campos *et al.* (2012), lysine is the second limiting amino acid, after the sulfur amino acids in poultry avian diets. However, high lysine intakemay negatively influence amino acid balance, reducing feed intake and increasing amino acid excretion. Therefore, the dietary balance between lysine and arginine is essential.

Arginine is synthetized from glutamate and ornithine, which is produced in the liver of mammals as part of the urea cycle (Lehninger *et al.*, 1995). On the other hand, poultry are not able to synthetize ornithine from glutamate because they lack the enzymespyrroline-5-carboxylatesynthase (converts glutamate to glutamate-5-phosphate) and ornithine-aminotransferase (transfers the amine grouping of glutamate for glutamate-5-phosphate to produce ornithine) and cannot convert ornithine in citrulline because of the absence of ornithine-transcarbamylase (responsible for converting carbamoyl phosphate and ornithine into citrulline) which prevents the occurrence of the urea cycle in birds. Therefore, poultry arginine requirements are much higher relative to mammals (Bacila, 2003). In addition, because poultry diets are based on corn and soybean meal, where arginine is considered limiting, this amino acid must be supplied (Edmonds *et al.*, 1985).

Moreover, there are clear evidences of considerable antagonism between arginine and lysine. When L-amino acids with similar chemical structure are unbalanced in the diet, the urinary excretion of amino acid at with the lowest concentration increases (Nunes, 1998). The excess of lysine may reduce the activity of the enzyme glycine-amidino transferase in the liver, and thereby may limit the production of creatine. Excessive lysine levels in poultry diets may lead to competition of lysine with arginine for kidney tubular reabsorption, because both amino acids have the same absorption site, which may lead to increasing kidney arginase activity, which oxidizes arginine in ornithine and urea. This demonstrates that high levels of dietary lysine increase arginine requirement (Macari *et al.*, 2002). In experiments to determine lysine requirements, arginine should be the limiting amino acid in order to maintain the maximum expression of the genetic potential by chickens (Gadelha *et al.,* 2003).

The National Research Council (NRC, 1994) recommendation for laying hens is 0.7% total arginine for a feed intake of 100 g/hen/day and 0.88% for a feed intake of 80 g/bird/day. Rostagno *et al.* (2011) suggested level of 0.866% of digestible arginine for a feed intake of 93 g/hen/day, 0.804% for a feed intake of 100 g/hen/day, and 0.748% for a feed intake of 108 g/hen/day layers. The Lohmann LSL manual for white layers (Lohmann LSL, 2004) makes recommendations according to production phase and not to feed intake, with 1.08% total arginine for 90% of lay, 0.99% total arginine for 89-80% of lay, and 0.95% total arginine for less than 80% of lay.

In this context, the optimal levels of arginine and lysine need to be determined in order to prevent the antagonism between these amino acids, which may negatively affect egg quality.Therefore, this study aimed at evaluating the influence of the dietary digestible lysine and digestible arginine supplementation at different ratios on the egg quality of laying hens between 24 and 44 weeks of age.

MATERIAL AND METHODS

The experiment was carried out in the facilities of the Federal University of Goiás, Goiânia, Brazil. A total number of 320 24-week-old Lohmann LSL layers were housed in 40 conventional cages (40x100x42 cm) in an experimental laying house with concrete floor, at eight hens per cage (two per pen). The cages were equipped with nipple drinkers and individual trough feeders. Hours of light were gradually increased until the on set of lay, and reached 17 hours at the peak of lay (34 weeks of age). An automatic timer to control the lighting program and 60-W lamps were used.

Diets were fed *ad libitum* twice a day, in the morning (8:00m) and afternoon (16:00m). Temperature and air humidity levels inside the experimental facilities were recorded daily at 8:00m and 17:00m using a thermo-hygrometer. Average minimum and maximum temperatures of 20.15 and 30.33ºC, and 69.1 and 87.2% average minimum and maximum relative humidity values were recorded.

A completely randomized design in a 2 x 4 factorial arrangement was applied, with eight treatments and four replicates of 10 hens each (five pens with two hens each). Two digestible lysine levels (700 or 900 mg/kg of diet) were combined with four digestible arginine levels (700, 800, 900, or 1000 mg/kg of diet), resulting in digestible lysine to digestible arginine ratios of 100, 114, 128 and 142 when the diet with 700 mg lysine was fed, and 78, 89, 100, and 111 when 900 mg lysine was fed.

The formulation of the experimental diets was based on the ingredient composition and nutritional requirements proposed by Rostagno *et al.* (2000). The diets contained 2800 kcal of metabolizable energy/kg of diet and 16% crude protein (Table 1). Lysine-HCl (0.178 and 0.202%) was added to diets to obtain 700 and 900 mg of digestible lysine per kg of diet, and 0.0, 0.105, 0.211, and 0.316%of L-Arginine to supply the proposed levels of 700, 800, 900, and 1000 mg of digestible arginine. Amino acids were added in replacement of cassava starch.

Five periods of 28 days were evaluated (from 24 to 44 week of age). During the last three days of each 28-day experimental period, a sample of 16 eggs per treatment was collected. Intact eggs, yolks and eggshells were weighed in a digital scale (Chyo Petit Balance, model MK 500 C, Moretti, Argentina) with 0.01g precision. Before weighing, the eggshell of each egg was washed and dried in a forced-ventilation oven at 55°C for 24 hours. Albumen weight was obtained by difference between egg weight and the weights of the eggshell and of the yolk. The weight of all egg components is expressed as percentages of total egg weight.

Yolk index was calculated as a quotient between yolk height, measured with a micrometer (Ames S-6428), and yolk diameter measured using a caliper. In order to determine Haugh units, eggs were broken on a plate and albumen height was measured using a micrometer (Ames S-6428). Haugh units were calculated according to the following logarithmic expression: UH = 100 log

Table 1 – Composition and calculated nutritional values of the basal diet.

Ingredient	(g/kg, as fed)
Corn	646
Soybean meal 42%	97
Wheat bran	55
Corn gluten 60%	55
Limestone	90
Meat meal	40
Salt	0.039
Mineral and vitamin supplementation*	0.012
L-Lysine HCL	0.017
L-Arginine HCl	0.000
DL-Methionine 99	0.016
L-Tryptophan	0.007
Starch	0.061
Total	100
Metabolizable energy (kcal/kg)	2,800
Crude protein (g/kg)	160
Calcium (g/kg)	40.5
Available phosphorous (g/kg)	3.8
Lysine (g/kg)	7.7
Digestibel lysine(g/kg)	7.0
Arginine (g/kg)	7.5
Digestible arginine (g/kg)	7.0
Methionine + cystine (g/kg)	6.9
Methionine (g/kg)	3.9
Threonine (g/kg)	5.9
Tryptophan(g/kg)	2.0
Arginine:Lysine	1.00

* Mineral and vitamin supplement (composition/kg of feed): Vit. A-2,500,000 IU, Vit. D3-625,000 IU, Vit.-E 3750 mg, Vit. K3-500 mg, B1-500 mg, B2-1000 mg, B6-1000 mg, B12-3,750 mcg, Niacin-7,500 mg, Acid pantohenate-4,000 mg, Biotin-15 mg, Folic acid-125 mg, Choline-75,000 mg, Selenium-45 mg, Iodine-175 mg, Iron-12,525 mg, Copper-2,500 mg, Manganese-19,500 mg, Zinc-13,750 mg, Avilamicin-20,000 mg.

$(H - 1.7W + 7.6)$, where H is dense albumen height (mm) and Wis egg weight (g).

Specific gravity was determined in eggs collected during the last two days of each 28-d period, by immersing eggs in buckets containing different saline solutions (NaCl), which densities ranged from 1.065 to 1.100 at 0.005 intervals, as calibrated with densitometer (Incoterm 5599). The specific saline solution in which the egg floated was individually recorded for all treatments.

In order to calculate total egg, yolk, and albumen solids, 12 eggs per treatment were broken and placed in individual recipients. Egg components were separated, weighed, and dried in a forced-ventilation oven at 65 ± 5°Cfor 72 hours and, after this, in an oven at 105°C for 24 hours, then the residues weighed.

For statistical analysis, theaverage ofthe data obtained in the five periods was calculated (from 24 to 44 week of age). The averages were then submitted to analysis of variance using the statistical program SAEG (Versão 7.1) at 5% probability level. The four levels of digestible arginine tested were submitted to polynomial regression analyses.

RESULTS AND DISCUSSION

No statistical interaction of dietary digestible lysine and digestible arginine supplementation were observed for average egg weight (Table 2). Therefore, the different digestible lysine: digestible arginine ratios evaluated in this experiment did not affect the weight of the eggs or of its components. However, Araújo *et al.* (2005), testing six digestible lysine: arginine ratios (718:716, 790:716, 718:644, 790:644, 718:788, and 790:788 mg/kg of diet) in Lohmann Brown and Lohmann LSL 40-week-old layers, obtained lower egg weight when the diet contained the ratio of 790:716 digestible lysine: arginine/kg of diet. Lima & Silva (2007) fed white and brown layers with diets containing two digestible lysine levels (710 or 780 mg/kg of diet) and three digestible arginine levels (640, 720, or 790 mg/kg of diet) and observed significant interaction effects. When layers were fed 720 mg digestible arginine/kg of diet, heavier eggs were obtained with the lowest digestible lysine level (710 mg/kg of diet). When the diet contained 780 mg digestible lysine/kg of diet, the heaviest eggs were obtained when 790 mg digestible arginine/kg of diet were fed. Those authors observed that digestible arginine levels must be adjusted as digestible lysine levels increase. This result was not observed in the present experiment because the increase in lysine and arginine dietary levels did not affect egg weight and the lowest digestible lysine and digestible arginine ratio (100) seemed to be sufficient to maintain egg quality.

Average egg weight, and albumen and yolk percentages were not influenced by the evaluated digestible lysine and arginine levelswhen individually analyzed (Table 2).

Increasing dietary digestible lysine levels have been previously studied (Silva *et al.*, 2010; Jardim Filho *et al.*, 2010) and the results indicated that higher lysine levels resulted in heavier eggs, higher albumen percentage (Jardim Filho *et al.*, 2008; Schmidt *et al.*, 2008; Silva *et al.*, 2010; Jardim Filho *et al.*, 2010), and higher yolk percentage (Schmidt *et al.*, 2008; Rocha *et al.*, 2009; Jardim Filho *et al.*, 2010; Silva *et al.*, 2010). Other authors found increased egg weight with 770

Table 2 – Egg quality evaluation for laying hens fed different levels of digestible lysine (Lys, mg/kg of diet) and arginine (Arg, mg/kg of diet) from 24 to 44 week of age.

	Egg weight (g)	Eggshell (%)	Albumen (%)	Yolk (%)
		Digestible lysine		
700	58.84	9.50 a	64.50	26.00
900	58.59	9.33 b	64.64	26.03
		Digestible arginine		
700	58.90	9.41	64.59	26.00
800	58.43	9.40	64.80	25.80
900	58.78	9.31	64.69	26.00
1000	58.76	9.52	64.20	26.28
		P value		
Lys	0.560	0.002	0.206	0.870
Arg	0.881	0.070	0.359	0.410
Lys x Arg	0.793	0.541	0.395	0.736
CV %	2.06	1.53	0.60	2.13

a, b – different letters in the same column are statistically different by the F test (5%).

mglysine/kg of diet (Rocha *et al.*, 2009) or a quadratic effect with a maximum point of 685 mg lysine /kg of diet (Schmidt *et al.*, 2008) .

In the present experiment (Table 2), lysine supplementation influenced eggshell quality ($p<0.05$), and the best eggshell percentage was obtained with 700 mg digestible lysine compared with 900 mg digestible lysine per kg of diet. This result may have been influenced by the higher intake of the layers fed the diets containing 700 mg of lysine (Carvalho *et al.*, 2012), which consequently increased calcium intake, increasing calcium availability for eggshell formation. The highest lysine supplementation level could potentially change the balance among essential amino acids, which, consequently, could interfere with the hens' metabolism. In this experiment, the antagonism between lysine and arginine was not observed. However, according to Montanhini Neto *et al*. (2013), the lysine: arginine dietary ratio may affect methionine requirements of broilers, influencing their growth. Higher arginine excretion requires more energy, particularly in uricotelic species, because its molecular structure contains the highest amount of nitrogen among amino acids (Lehninger *et al.*, 1995). Every uric acid molecule is excreted along with a molecule ofglycine. Although birds are able to synthetize glycine, its rate of synthesis is not sufficient speed to supply tissue metabolism needs and to eliminate nitrogen excess (Corzo *et al.*, 2004). Under these conditions, methionine can be limiting due to the higher metabolic requirements formethylation reactions (Silva *et al.*, 2012).

There was no statistical interaction between digestible lysine and arginine interaction for egg-quality parameters in this experiment (Table 3). The effects of different digestible lysine to digestible arginine ratios tested were not statistically significant. Reis *et al.* (2012) did not find any performance or egg quality differences when testing different arginine: lysine ratios (1.16, 1.21, 1.26, 1.31, and 1.36). On the other hand, Carvalho *et al.* (2012) obtained better performance and higher nutrient metabolizability with different digestible lysine to digestible arginine ratios and concluded that, in order to improve layer performance and to optimize nitrogen metabolism, increases in digestible lysine levels should be accompanied by digestible arginine supplementation.

When amino acid levels were studied separately, digestible lysine levels did not affect egg specific gravity or egg internal quality (Table 3). Other experiments showed that increasing the levels of digestible lysine of layer diets did not affect Haugh units (Jardim Filho *et al.*, 2008; Schmidt *et al.*, 2008; Matos *et al.*, 2009; Rocha *et al*, 2009; Jardim Filho *et al.*, 2010; Silva *et al.*, 2010;), yolk index (Jardim Filho *et al.*, 2008; Schmidt *et al.*, 2008; Rocha *et al*, 2009; Jardim Filho *et al.*, 2010), or total egg solids (Jardim Filho *et al.*, 2008; Matos *et al.*, 2009; Jardim Filho *et al.*, 2010).

The digestible arginine levels used in this experiment did not affect egg specific gravity or any egg internal quality parameters (Table 3), except for albumen solids ($p<0.05$), with a negative quadratic effect of reducing

Table 3 – Egg components of 24 – to 44 – week - old layers fed increasing levels of digestible lysine (Lys, mg/kg of diet) and arginine (Arg, mg/kg of diet).

	SG[1]	UH[1]	YI[1]	ETS[1]	AS[1]	YS[1]
			Digestible lysine			
700	1.091	102.16	0.43	23.60	11.79	50.78
900	1.091	102.76	0.43	23.30	11.85	50.82
			Digestible arginine			
700	1.093	102.34	0.43	23.46	12.11	50.83
800	1.090	102.48	0.43	23.39	11.65	50.73
900	1.090	102.73	0.43	23.33	11.72	50.61
1000	1.091	102.29	0.43	23.63	11.80	51.02
			P value			
Lys	0.964	0.129	0.759	0.072	0.484	0.763
Arg	0.282	0.847	0.639	0.567	0.002[2]	0.257
Lys x Arg	0.794	0.080	0.308	0.896	0.543	0.165
CV %	0.36	1.05	1.43	1.91	1.97	0.80

a, b – different letters in the same column statistically differ. [1] Specific gravity (SG), Haugh units (HU), yolk index (YI), whole egg solids (ETS), albumen solids (AS) and yolk solids (YS).

[2] $Y= 22.0930 – 0.0237007X + 0.0000134411X^2$ / $R^2= 0.89$ / minimum point = 881 mg

the content up to 881 mg digestible arginine/kg of diet. Rostagno *et al.* (2011) suggested that the level of 0.866% of digestible arginine for a feed intake of 93 g/hen/day is close to the observed in this experiment, which was 94.7 g/hen/day.

There was no interaction between digestible lysine and arginine levels for egg quality parameters. Increasing the dietary levels of digestible lysine and arginine reduced eggshell quality and albumen solids, respectively.

The levels of amino acids suggested to improve egg quality are 700 mg of digestible lysine and 700 mg of digestible arginine/kg of feed, corresponding to a digestible lysine: digestible arginine ratio of 100.

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