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Optimal Standardized Ileal Digestible Arginine to Lysine Ratio for Japanese Quails in the Egg-Laying Phase

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ABSTRACT

This study was conducted to determine the optimal standardized ileal digestible arginine to lysine (SID Arg:Lys) ratio for Japanese quails in the egg-laying phase. A total of two hundred forty-five 35-week-old Japanese quails (181 ± 1.30 g initial body weight) were randomly assigned to five treatment groups with seven replicates of seven quails. Graded levels of L-Arginine were added to a basal diet in order to produce five SID Arg:Lys ratios (101, 106, 111, 116 and 121%). Collected data were analyzed as one-way ANOVA and optimal ratio was estimated using polynomial regression model (linear and quadratic) based on performance traits. Statistical differences were considered for $p<0.05$. Graded SID Arg:Lys ratios did not affect performance traits assessed. Based on results, the SID Arg:Lys ratio of 101% is sufficient to warrant proper performance of Japanese quails in the egg-laying phase.

INTRODUCTION

Poultry are not able to synthetize arginine (Arg) themselves, and therefore, supplying adequate amounts of dietary Arg is essential to meet the bird nutritional requirements (D'Mello & Lewis, 1971). Beyond playing its classical role of building blocks for body and feather protein synthesis, Arg participates on the biosynthesis of several molecules, such as creatine and polyamines (Khajali & Wideman, 2010). In avian species, Argis involved in reproductive physiology (Grossman *et al.*, 2002), since it is one of the components of avian neurohypophysial peptide arginine vasotocin, which among other functions, influences reproductive tract physiology by stimulating shell gland contractility and oviposition (Koike *et al.*, 1988). Moreover, Arg participates in the pathway of nitric oxide synthesis (Jobgen *et al.*, 2006), which modulates reproductive functions in poultry (Sundaresan *et al.*, 2007 and Kumar & Chaturvedi, 2008), regulating follicular development, ovulatory mechanisms, and egg production (Manwar *et al.*, 2006).

Few is known about how limiting Arg is in diets compared with other essential amino acids, and contrary to lysine, methionine, threonine, valine and tryptophan, Arg is not available as afeed grade amino acid trade product. Perhaps due to such reason, few are the research efforts put forward establishing either poultry requirements for Argor its ideal relative ratio to lysine. Protein is widely recognized as the most expensive nutrient in poultry diets, though nutritionists tend to reduce protein content in practical diets with the purpose of saving feed cost, which, in some cases, may limit essential amino acid supply. Crystalline amino acid supplementation in low-protein diets offers several advantages in poultry production, such as reducing feed costs and nitrogen excretion in the environment (Franco *et al.*, 2016). However, as aforementioned, the industrial production of feed-grade amino acids is limited to only few amino acids, which frequently limit further reductions of dietary crude protein.

Establishing Japanese quail requirements for all essential amino acids would contribute to meet more precisely nutritional requirements. Since proposed, the ideal protein concept has been the most commonly used approach to formulate poultry diets, which must supply all amino acids at optimal ratios relative to dietary Lys content. Literature reports optimal SID Arg:Lysratios for Japanese quails of 116% (Rostagno *et al.*, 2011), 132% (Silva & Costa, 2009), and 126% (NRC, 1994). Although such nutritional recommendations are important references, further studies are needed to update the nutritional requirements of Japanese quails. Reis *et al.*, 2012 and Maurício *et al.*, 2016 demonstrated that the optimal SID Arg:Lys ratios for Japanese quails are lower than the recommendations mentioned above. Therefore, this study was conducted to determine the optimal SID (standardized ileal digestible) Arg:Lys ratio for Japanese quails in the egg-laying phase.

MATERIALS AND METHODS

All the procedures described below were previously approved by the institutional Animal Care and Use Committee of the Universidade Federal de Viçosa, Minas Gerais, Brazil (protocol number 38/2012).

Husbandry, diets and experimental design

A total of 245 35-to-44-week-old Japanese quails were selected from a local commercial flock according to body weight (181 ± 1.30 grams), and randomly allocated to five treatments with seven replicates of seven birds each. Birds were housed in 25x 35 x 15 cm (length x width x height) stainless steel cages, equipped with an automatic feeder and a nipple drinker. Photoperiod was set at 16L:8D throughout the 9-week feeding trial, in which quails had free access to water and feed (mash form).Diets were formulated to be isonitrogenous (175.50 g CP/kg diet) and isocaloric (2,800 kcal ME/kg diet). All other nutrients were supplied to meet or exceed Rostagno *et al.* (2011) recommendations. The experimental treatments consisted of five diets with graded SID Arg:Lys ratios (101, 106, 111, 116,or 121%), which were obtained by the graded supplementation of L-Arginine (0.00, 0.51, 1.01, 1.52 and 2.02 g/kg diet) at the expense of L-Glutamic acid. The nutritional composition of the feed stuffs used to formulate basal diet were assumed to be as described by Rostagno *et al.* (2011). Crystalline amino acids were assumed to be 100% digestible. Since the objective of the current study was to determine Arg requirement as a ratio of Lys requirement, the basal diet was formulated to be first limiting in Arg and second limiting in Lys (Boisen, 2003). The suboptimal level of Lys chosen to formulate the basal diet corresponded to 92% of the level recommended by Rostagno *et al.* (2011).

Performance and egg quality measurements

All the eggs produced were daily recorded, whereas other performance and egg quality traits

Table 1 – Ingredients and calculated composition of the experimental diets on as-fed basis.

	SID Arg:Lys, %				
Item	101	106	111	116	121
Ingredient, g/kg					
Corn	359.51	359.51	359.51	359.51	359.51
Soybean meal	238.82	238.82	238.82	238.82	238.82
Sorghum	260.00	260.00	260.00	260.00	260.00
Wheat bran	27.50	27.50	27.50	27.50	27.50
Soybean oil	12.61	12.61	12.61	12.61	12.61
Limestone	68.94	68.94	68.94	68.94	68.94
Dicalcium phosphate	11.14	11.14	11.14	11.14	11.14
Salt	3.33	3.33	3.33	3.33	3.33
Glutamic acid	7.00	5.31	3.68	2.03	0.36
Corn starch	1.00	2.18	3.31	4.46	5.62
L-Lysine HCl (79%)	2.89	2.89	2.89	2.89	2.89
DL-Methionine (99%)	3.71	3.71	3.71	3.71	3.71
L-Threonine (98%)	0.42	0.42	0.42	0.42	0.42
L-Valine (99%)	0.35	0.35	0.35	0.35	0.35
L-Tryptophan (99%)	0.18	0.18	0.18	0.18	0.18
L-Arginine (99%)	0.00	0.51	1.01	1.51	2.02
Premix[1]	2.00	2.00	2.00	2.00	2.00
Choline chloride	1.00	1.00	1.00	1.00	1.00
Calculated composition					
Metabolizable energy, kcal/kg	2,800	2,800	2,800	2,800	2,800
Crude protein, g/kg	175.50	175.50	175.50	175.50	175.50
Calcium, g/kg	30.99	30.99	30.99	30.99	30.99
Non-phytate phosphorus, g/kg	3.23	3.23	3.23	3.23	3.23
Sodium, g/kg	1.55	1.55	1.55	1.55	1.55
Digestible amino acids, g/kg					
Lysine	10.00	10.00	10.00	10.00	10.00
Arginine	10.10	10.60	11.10	11.60	12.10
Methionine + cysteine	8.20	8.20	8.20	8.20	8.20
Threonine	6.00	6.00	6.00	6.00	6.00
Valine	7.50	7.50	7.50	7.50	7.50
Tryptophan	2.10	2.10	2.10	2.10	2.10

[1]Provided per kilogram of diet: vitamin A, 7,500 IU; vitamin D_3, 2,000 IU; vitamin E, 10 IU; vitamin K, 1.80 mg; vitamin B_1, 1.5 mg; vitamin B2, 4.0 mg; nicotinic acid, 25 mg; pantothenic acid, 10 mg; vitamin B6, 1.7 mg; vitamin B12, 0.013 mg; folic acid, 0.50 mg; biotin, 0.05 mg; copper, 11 mg; iron, 55 mg; iodine, 1.10 mg; manganese, 77 mg; selenium, 0.33 mg; zinc, 72 mg and butylated hydroxytoluene, 0.1 g.

were measured at three 21-day intervals. Average daily feed intake (ADFI) was measured every 21 days. All the eggs laid with no eggshell abnormalities were weighed on the last 3 days of each 21-day interval in order to obtain average egg weight. Egg mass and feed conversion ratio (FCR) per kilogram of eggs were calculated based on egg weight. Mortality was daily recorded, and used to adjust ADFI, FCR and egg production results. Specific egg weight and egg components were measured during the last 3 days of each 21-day interval. Specific egg weight was determined by immersing the eggs in graded saline solutions (1,050-1,100 g/cm^3), as described by Thompson and Hamilton *et al.* (1982). Eggs were then broken to determine egg component values. The yolk was separated from the albumen and weighed. Eggshells were washed, dried (at 55-65°C), and weighed. Albumen weight was calculated as the difference between total egg weight and the sum of yolk weight and eggshell weight. All egg component weights were expressed in g and their weight relative to egg weight (i.e., yield) was calculated.

Statistical analysis

Each cage housing seven quails was considered as an experimental unit for statistical analysis. Data were analyzed as one-way ANOVA using the statistical software SAEG (SAEG, 2007). The optimal SID Arg:Lys ratio for each performance trait assessed was estimated by polynomial (linear or quadratic) regression model. Statistical significance was considered at $p<0.05$.The statistical model considered experimental treatment as fix effect: Yik = μ + SID Arg:Lys$_i$+ eik. Where Y_{ik} = dependent variable; μ = overall mean; SID Arg:Lys$_i$ = standardized ileal digestible arginine to lysine ratios (i = 101, 106, 111, 116 and 121%); e_{ik} = random error term.

RESULTS AND DISCUSSION

The evaluated SID Arg:Lys ratios (101, 106, 111, 116 and 121%)did not affect (p>0.05) either performance (Table 2) or egg quality traits assessed (Table 3). Our outcomes are in agreement with Reis *et al.* (2012), who did not observe any effects of SID Arg:Lys ranging between 116 and 136% on the performance and egg quality traits of Japanese quails. This finding suggests that the optimal SID Arg:Lys ratio required by Japanese quails in the egg-laying phase is lower than ratios evaluated herein, and lower than those recommended by Rostagno *et al.* (2011) (116%), Silva & Costa (2009) (132%) and the NRC (1994) (126%). Similarly, Santos (2010) did not report any performance or egg quality differences in Japanese quails fed 82% or152% Arg:Lys ratios. Therefore, although the outcomes of those dose-response studies indicate the minimal SID Arg:Lys ratio studied as optimal, further research must be conducted. The establishment of the minimum amount of Arg required to meet maintenance purposes and egg protein synthesis demand could contribute to

Table 2 – Production performance of Japanese quails in lay fed increasing SID Arg:Lys ratios.

Item	SID Arg:Lys ratios (%)					Effect		SEM[3]
	101	106	111	116	121	L[1]	Q[2]	
ADFI* (g/bird/day)	25.65	26.40	26.22	26.26	25.77	0.99	0.19	0.45
Egg production (%)	84.89	85.67	84.89	85.89	82.84	0.99	0.16	3.05
Egg weight (g)	11.49	11.47	11.55	11.69	11.53	0.99	0.15	0.48
Egg mass (g/bird/day)	9.75	9.82	9.80	10.3	9.54	0.06	0.32	0.43
FCR* (kg/dozen eggs)	0.364	0.371	0.372	0.367	0.374	0.13	0.99	0.01
FCR (kg/kg)	2.64	2.69	2.68	2.62	2.71	0.11	0.99	0.11

*ADFI: average daily feed intake; FCR: feed conversion ratio. [1]Linear effect. [2]Quadratic effect. [3]Standard error of the mean.

Table 3 – Egg quality traits of Japanese quails in lay fed increasing SID Arg:Lys ratios.

Item	SID Arg:Lys ratios (%)					Effect		SEM[3]
	101	106	111	116	121	L[1]	Q[2]	
Albumen weight (g)	7.24	7.27	7.26	7.46	7.27	0.99	0.99	0.15
Albumen percentage (%)	62.07	62.15	61.82	62.74	62.17	0.99	0.15	1.17
Yolk weight (g)	3.50	3.49	3.55	3.51	3.49	0.99	0.99	0.08
Yolk percentage (%)	30.04	29.84	30.32	29.55	29.86	0.99	0.99	0.67
Eggshell weight (g)	0.92	0.94	0.92	0.92	0.93	0.99	0.99	0.03
Eggshell percentage (%)	7.89	8.01	7.86	7.71	7.97	0.99	0.19	0.23
Specific gravity (g/cm^3)	1.073	1.074	1.073	1.072	1.073	0.99	0.99	0.01

[1]Linear effect. [2]Quadratic effect. [3]Standard error of the mean.

formulate diets with a lower crude protein content, resulting reductions in feed cost and nitrogen excretion.

Amino acid requirements are strongly influenced by sex, age, genetic strain, environmental temperature and health status. However, feedstuffs included in diet formulation and some nutrients may also affect amino acid requirements. In studies conducted to determine poultry requirements for Arg, dietary Lys content must be considered, since antagonisms between both amino acids affect requirements (Lewis *et al.*, 1963; D'Mello & Lewis, 1970). Dean and Scott (1968) reported that broiler performance was impaired due to excessive Lys intake. According to the same authors, extra crystalline Arg supplementation in high-Lys diets alleviated such detrimental effects. Arginine and Lys are basic amino acids, and therefore, share the same intestinal uptake system (Closs & Mann, 2000). Considering that Lys concentration affects Arg absorption (D'Mello & Lewis, 1970), excessive Arg content may also limit Lys uptake. In the current study, it was observed that increasing Arg:Lys ratios did not compromise the performance or egg quality traits of Japanese quails. However, contrary to our findings, Maurício *et al.* (2016) observed a linear decrease egg production when Japanese quails were fed SID Arg:Lys ratios ranging from 106 to 146%, showing detrimental effects of excessive Arg supply on the performance, and that a 106% SID Arg:Lys ratio warrants proper quail performance.

Impairments in performance of birds fed high dietary Lys levels may be also attributed to changes in the activity of the enzymes involved in Arg metabolism. The most classical example of lysine-arginine antagonism is the increase in kidney arginase activity, resulting in Arg catabolism (Silva *et al.* 2012). Excessive Lys has been also shown to influence the activity of arginine:glycine amidinotransferase (AGAT; Silva *et al.* 2012), which is involved in the synthesis of guanidinoacetic acid (GAA) in poultry liver. Guanidinoacetic acid is required in creatine biosynthesis pathway (Jung *et al.* 2013). Its role in the nutrition of Japanese quails in the egg-laying phase was recently reported by Murakami *et al.* (2014), who observed a linear increase in GAA and creatine contents in the egg of meat-type quail breeders in response to increasing GAA supplementation. Although breeder performance was not affected by GAA, the aforementioned authors observed improvement the performance of the offspring of GAA-supplemented breeders.

Considering the interactions between Lys and Arg, Lys levels different from that supplied in the current study may produce different estimates of the ideal SID Arg:Lys ratio. Despite not establishing the optimal SID Arg:Lys ratio for Japanese quails, our results support the hypothesis that the optimal SID Arg:Lys ratios reported in literature for Japanese quails are excessive, and therefore maybe reduced. Hence, crude protein could be potentially reduced, resulting in benefits like the decrease in feed cost and the mitigation of nitrogen excretion in environment. We suggest that further studies be designed with lower levels of Arg than the studied herein with a wide range between the lower and higher levels under study. Based on the performance and egg quality results, SID Arg:Lys ratio of 101% is sufficient to warrant proper performance and egg quality of Japanese quails in the egg-laying phase.

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