



Performance and egg quality of laying Japanese quails (*Coturnix coturnix japonica*) reared in hot climate as a function of digestible arginine: lysine ratios in the diet

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ABSTRACT

The objective of this study was to determine the ideal digestible arginine: lysine ratio in the diet of laying Japanese quails reared in hot climate, on the variables of productive performance and egg quality. Completely randomized experimental design was adopted with five diets and six replications containing eight laying quails (*Coturnix japonica*) per experimental unit, totaling 240 birds with an average weight of 169.6±0.005 g and 40 weeks of age with an initial egg production of 79.18±3.32%. The digestible arginine: lysine ratios were 110%, 120%, 130%, 140% and 150%. The arginine: lysine ratios analyzed were not able to influence feed intake, egg production, commercial eggs, egg weight, egg mass, feed conversion per egg mass (FCEM) and per dozen eggs (FCDZ); however, there was a quadratic effect for the variation in body weight. There was no significant difference for egg quality characteristics. The 110% digestible arginine: lysine ratio in the diet of laying Japanese quails reared in a hot climate is sufficient to maintain the productive performance and the egg quality.

Keywords: Amino acids, Antagonism, Body weight, Feed efficiency, Ideal protein, Laying quail

Of the components of the diet, protein is recognized as the more expensive nutrient. Although the reduction of crude protein as a nutritional strategy to minimize costs is widely used, in most cases, the requirements for essential amino acids are not met. Thus, the use of the concept of ideal protein, together with the supply of essential amino acids in poultry nutrition has been the object of study by several researchers (Hernandez *et al.* 2012, Gheisari *et al.* 2015) and offers numerous advantages such as increased protein retention efficiency, lower excretion of nitrogen and uric acid, reduced caloric increase, reduced food costs (Applegate *et al.* 2008) and production with the lowest possible environmental impact.

Arginine plays a fundamental role in body and feather protein synthesis, and contributes to the biosynthesis of various molecules, such as creatine and polyamines (Khajali and Wideman 2010). In addition, the participation of arginine in the nitric oxide synthesis pathway (Jobgen *et al.* 2006) and the activity in the modulation of reproductive functions (Sundaresan *et al.* 2007) such as follicular development, ovulatory mechanisms, and egg production

(Manwar *et al.* 2006), makes this amino acid extremely important in the productive performance of laying birds.

The birds do not have a functional urea cycle due to the absence of the enzyme carbamoyl-phosphate synthase, which has the function of catalyzing the first reaction of the urea cycle in which ammonia and CO₂ are involved. Thus, supplementation is necessary due to the greater requirement of this essential amino acid in poultry diets (Fouad *et al.* 2013). In some cases, antagonism with arginine in diets with excess lysine is common, generating arginine deficiency due to urinary losses caused by competition with lysine for renal tubular reabsorption and increased renal arginase enzyme, promoting catabolism arginine in the body (D' Mello 2003).

The recommendations of the arginine: lysine ratio for Japanese quails in the production phase (132%, Silva and Costa 2009; 115%, Rostagno *et al.* 2017 and 101%, Tuesta *et al.* 2018) are scarce in the literature, showing a discrepancy between the recommendations and needs to be updated. Therefore, the objective of this study was to determine the ideal digestible arginine: lysine ratio in the diet of laying Japanese quails reared in hot climate, on the variables of productive performance and egg quality.

MATERIALS AND METHODS

The experiment was carried out in the Coturniculture sector of the Experimental Farm of the Federal University

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of Mato Grosso, Brazil. The project was approved by the Animal Use Ethics Committee of the University under the protocol: 23108.042093/2020-07.

Diets, animals and experimental design: A total of 240 laying quails (*Coturnix coturnix japonica*) were selected, weighing 169.6 ± 0.005 g at 40 weeks of age with an initial egg production of $79.18 \pm 3.32\%$. A completely randomized design was used with five diets and six replications containing eight quails per experimental unit. The studied digestible arginine: lysine ratios were 110%, 120%, 130%, 140% and 150% for 63 days divided into three periods of 21 days each.

The experimental diets (Table 1) were formulated based on corn and soybean meal, being isoenergetic and isoproteic, according to the recommendations and nutritional compositions of Rostagno *et al.* (2017) for laying Japanese quails and Lima (2018) for the requirements of available phosphorus, calcium, sodium, protein and energy. The composition of the ration was calculated using

samples from the diets of each treatment and analyzed in triplicate for the contents of dry matter, mineral matter, crude protein, crude fibre, ether extract, phosphorus, and calcium according to CBAA (2017).

The diets were formulated by supplementing L-Arginine (99%) (0; 0.1; 0.2; 0.3; 0.4 g/kg), replacing glutamic acid, in protein equivalent, corresponding to the arginine: lysine ratios of the respective treatments (110, 120, 130, 140, 150%), at the total arginine levels of 1.11; 1.21; 1.31; 1.41 and 1.51 g/kg feed. The differences resulting from the balance for the energy equivalents of arginine and glutamic acid were offset by starch.

The birds were housed in 30 galvanized wire cages, horizontally arranged, with the dimensions of $50 \times 38 \times 21$ cm (length \times width \times height), provided with trough type and nipple type water trough, constituting an area of 190 cm²/bird for each experimental plot. Feeding and recording of ambient temperatures and relative humidity were carried out twice a day (8 and 16 h), and water was provided

Table 1. Ingredients and percentage composition (analyzed and calculated) of the laying Japanese quail diets with different arginine: lysine ratios

Ingredient (%)	Arginine: lysine ratio (%)				
	110	120	130	140	150
Corn	54.46	54.46	54.46	54.46	54.46
Soybean meal (45%)	28	28	28	28	28
Soybean oil	2.5	2.5	2.5	2.5	2.5
Limestone	9	9	9	9	9
Dicalcium phosphate, DCP	1.3	1.3	1.3	1.3	1.3
Salt	0.21	0.21	0.21	0.21	0.21
Glutamic acid	0.3	0.25	0.2	0.15	0.1
L-Lysine HCl (78,84)	0.22	0.22	0.22	0.22	0.22
DL-Methionine (99%)	0.38	0.38	0.38	0.38	0.38
L-Threonine (98%)	0.04	0.04	0.04	0.04	0.04
L-Tryptophan (99%)	0.03	0.03	0.03	0.03	0.03
L-Arginine (99%)	0	0.1	0.2	0.3	0.4
Vitamin and mineral supplement ¹	2	2	2	2	2
Corn starch	1.56	1.51	1.46	1.41	1.36
<i>Nutritional composition analyzed and calculated</i>					
Metabolizable energy (kcal/kg)	2800	2800	2800	2800	2800
Crude protein (%)	17.55	17.55	17.55	17.55	17.55
Crude fibre (%)	2.45	2.45	2.45	2.45	2.45
Calcium (%)	4.00	4.00	4.00	4.00	4.00
Available phosphorus (%)	0.35	0.35	0.35	0.35	0.35
Sodium (%)	0.10	0.10	0.10	0.10	0.10
<i>Digestible amino acids (%)</i>					
Digestible lysine	0.99	0.99	0.99	0.99	0.99
Digestible arginine	1.11	1.21	1.31	1.41	1.51
Digestible methionine + Cystine	0.87	0.87	0.87	0.87	0.87
Digestible threonine	0.62	0.62	0.62	0.62	0.62
Digestible tryptophan	0.21	0.21	0.21	0.21	0.21

¹Vitamin-mineral supplement composition: Calcium (min) 80 g/kg, Calcium (max) 100 g/kg; Phosphorus (min) 37 g/kg, Sodium (min) 20 g/kg, Methionine (min) 21.5 g/kg, Lysine (min) 18 g/kg, Vitamin A (min) 125000 IU/kg, Vitamin D₃ (min) 25000 IU/kg, Vitamin E (min) 312 IU/kg, Vitamin K₃ (min) 20 mg/kg, Vitamin B₁ (min) 20 mg/kg, Vitamin B₂ (min) 62.5 mg/kg, Vitamin B₆ (min) 37.5 mg/kg, Vitamin B₁₂ (min) 200 mcg/kg, Folic acid (min) 6,25 mg/kg, Pantothenic acid (min) 125 mg/kg, Biotin (min) 1.25 mg/kg, Choline (min) 1700 mg/kg, Niacin (min) 312 mg/kg, Copper (min) 125 mg/kg, Iron (min) 680 mg/kg, Iodine (min) 8.75 mg/kg, Manganese (min) 937 mg/kg, Selenium (min) 3.75 mg/kg, Zinc (min) 500 mg/kg, Fluorine (max) 370 mg/kg.

ad lib. Throughout the experimental period, 16 h of daily light (natural + artificial) were provided.

Performance production and egg quality: At the beginning and end of each production cycle, quails and feed were weighed, and eggs were collected daily to measure performance variables viz. bodyweight variation (obtained by the difference between the initial weight and the final weight of the quails (g/bird)); feed intake (g/bird/day, calculated by the difference between the amount of feed provided and the leftovers); feed conversion ratio per egg dozen (kg/dz, calculated by the ratio of total feed consumption in kilograms divided by the dozen eggs produced). The total egg mass (g/bird) was calculated by multiplying the average egg weight (g) by the total number of eggs produced in the period. Subsequently, the feed conversion ratio per egg mass was obtained by the consumption of feed in kilograms divided by the total mass of eggs (g/g).

The egg production (%) was obtained using the number of eggs produced intact, including non-tradable, broken, cracked and abnormal eggs (eggs with a soft shell and without shell) being expressed as a percentage of the average of birds of the period. The percentage of commercial eggs (%) was calculated using the number of whole eggs over the total number of eggs harvested, multiplied by 100. The egg quality analyses were performed on the last three days of each 21-day period, with all intact eggs from each repetition being collected, from which three eggs were randomly selected.

The specific gravity (g/cm³) was calculated by immersing the eggs in saline solutions with a density ranging from 1.060 to 1.095 g/cm³, with an interval of 0.005 g/cm³, duly calibrated using a densimeter (OM-5565, Incoterm). The collected eggs were broken and the yolk weight (g) results were obtained by separating the yolks manually and weighing on a precision scale of 0.01 g. Then, all the shells were weighed on a 0.01 g precision scale, after washing the shell and drying it in the air for 72 h, obtaining the results of shell weight (g). The albumen weight (g) was obtained by the difference between the egg and shell weight and the yolk weight. In the end, the components of eggs albumen (%), yolk (%) and shell (%) were expressed in percentage.

The height of the yolk and albumen (mm) was calculated by the average value of the four measurements, by reading at four different points in the equatorial region using an external micrometer. The diameter of the yolk was obtained by a digital caliper with a precision of 0.01 mm. The yolk index calculation was performed using the formula:

$$YI = YH / YD$$

where YI, yolk index; YH, yolk height; YD, yolk diameter. Haugh unit was obtained by the formula:

$$HU = 100 \times \log (AH + 7.57 - 1.7 \times EW \times 0.37)$$

where AH, albumen height (mm) and EW, egg weight (g) according to Eisen *et al.* (1962).

Shell thickness (mm), including membranes, was calculated by averaging three different points. Measurements were made at the two poles and in the lateral

region of the egg with a digital caliper, with an accuracy of 0.01 mm. The shell weight per unit of surface area was calculated using the formula (Abdallah *et al.* 1993)

$$SWUSA = \frac{3.9782 \times (EW \times 0.7056)}{SW} \times 100$$

where EW, egg weight (g) and SW, shell weight (mg/cm²).

Statistical analysis: All data obtained were analyzed using the statistical software R (R Studio 2018), and the normality of the residues was verified through the Shapiro-Wilk test and the homogeneity of variances by the Bartlett test. To check the effects (P<0.05) of the treatments, results of *p* less than 0.05 were considered.

The following statistical model was adopted:

$$Y_{ik} = \beta_0 + \beta_1 + \epsilon_{ik}$$

where Y_{ik} , variable measured in experimental unit_k, fed with a diet containing arginine: lysine ratio; β_0 , general constant; β_1 , effect of the arginine: lysine ratio; ϵ_{ik} , random error associated with each observation. Subsequently, the effects of the different arginine: lysine ratios were estimated by means of analysis using linear and quadratic regression models, according to the best adjustment obtained for each variable.

RESULTS AND DISCUSSION

The average maximum and minimum ambient temperatures recorded during the experimental period were 34.64±3°C and 25.15±1°C in the morning, and 38.61±3°C and 30.13±4°C in the afternoon, respectively. The average maximum and minimum relative air humidity recorded were 55.77±29% and 30.13±15% in the morning, and 45.00±25% and 23.05±10% in the afternoon, respectively.

The maximum temperature, regardless of the period of the day, remained above the thermal comfort zone, which should be around 24°C for adult laying quails, ensuring better internal characteristics of egg quality and blood chemical parameters (El-Tarabany 2016). Environmental temperatures above the thermoneutrality zone, cause changes in the neuroendocrine physiology of birds and continuous activation of the hypothalamic-pituitary-adrenal axis, resulting in increased circulating levels of corticosterone, producing immunosuppression, hyperglycemia, greater protein catabolism and susceptibility to infections (Mehaisen *et al.* 2017).

In response, thermoregulatory mechanisms that include vasodilation, increased surface evaporative losses and respiratory tract (Paulino *et al.* 2019) are activated. Moreover, reducing feed intake is one of the main responses of laying birds to maintain homeothermic balance and minimize heat production (Attia *et al.* 2016). In the end, weight gain and productive performance are reduced in thermally stressed laying birds.

However, in this study, the different arginine: lysine ratios analyzed, were not able to influence the performance variables viz. feed intake, egg production, commercial eggs, egg weight, egg mass, FCEM, and FCDZ (P>0.05) (Table 2). Therefore, it was expected that egg mass, FCEM and FCDZ would not be affected by the different

Table 2. Productive performance of laying Japanese quails reared in hot climate as a function of digestible arginine: lysine ratios in the diet

Variable	Arginine: lysine ratio (%)					SEM	p value ¹
	110	120	130	140	150		
Daily feed intake (g/bird/day)	26.52±2.01	27.34±1.52	27.88±2.40	27.60±2.39	27.48±1.82	0.026	0.5692
Body weight variation (g/bird)	22.01±5.81	31.50±2.02	33.25±6.41	25.48±5.03	-13.16±4.11	0.003	<0.001
Egg production (%)	81.79±2.89	81.42±2.05	82.08±2.31	81.54±3.54	81.95±3.93	0.851	0.9992
Commercial eggs (%)	80.96±6.77	80.68±6.25	81.32±6.59	80.66±7.68	81.04±5.69	0.133	0.9919
Average egg weight (g)	10.66±0.48	10.96±0.47	10.88±0.47	10.84±0.36	10.86±0.58	0.061	0.6546
Egg mass (g/bird)	8.729±0.88	9.045±1.04	9.189±1.05	8.831±0.91	8.980±0.46	0.113	0.7482
FCEM (g/g)	2.991±0.35	3.053±0.44	3.192±0.49	3.163±0.47	3.087±0.30	0.053	0.7761
FCDZ (kg/dz)	0.391±0.04	0.393±0.04	0.414±0.05	0.411±0.06	0.399±0.03	0.006	0.7121

FCEM, feed conversion ratio per egg mass; FCDZ, feed conversion ratio per egg dozen. SEM, Standard error of the mean.

¹Values >0.05 are considered non-significant.

ratios. Even at high temperatures, the average feed intake obtained in this study (27.37 g/bird/day) remained similar (26.39 g/bird/day) to that found by Mauricio *et al.* (2016) using five arginine: lysine ratios in quail diets raised under an average temperature of 24.3±5.6°C.

There was a significant difference in body weight ($P<0.05$). The variation in body weight behaved in an increasing way until the arginine: lysine ratio was estimated at 124.8%, where the highest average weight gain was observed (36.17 g/bird). Quails that received higher ratios than 124.8% in the diet, had a decreasing weight gain until

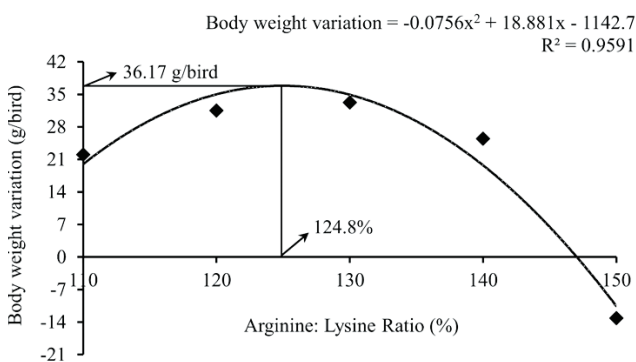


Fig. 1. Influence of arginine: lysine ratios on the variation in body weight of laying Japanese quails reared in a hot climate

they lost weight with the use of the highest ratio (150%) arginine: lysine (-13.16 g/bird), as shown in Fig. 1.

This result may be associated with higher energy expenditure for the excretion of nitrogen from excess amino acids since feed intake remained the same for all treatments. In a study by Atencio *et al.* (2000), in order to determine the requirement of digestible arginine and the ratio of digestible arginine: lysine to chickens, the authors observed that the diet with the highest level of digestible arginine showed the worst result for weight gain and feed conversion, probably due to the high level of arginine in the diet.

The effects not observed in this study in relation to weight and egg production reinforce the adequate isoproteic supplementation of all diets since the intake of proteins to supply the requirement of this nutrient in birds is linked to the size of the egg. Thus, it is possible to use the concept of

ideal protein, with reduced crude protein values in the diet, as observed in this study, using 17.55 g/kg crude protein in the diet, which is lower than the recommendation (18.92 to 19.07 g/kg) by Rostagno *et al.* (2017).

Another indication that protein supplementation was adequate in the studied diets is the lack of effects on the Haugh unit and albumen weight since, in a previous study, Maciel *et al.* (2019) demonstrated that diets with crude protein reduction supplemented with essential and non-essential amino acids meeting the requirements of laying Japanese quails, would have the characteristics of albumen equivalent to conventional diets of high crude protein.

The studied arginine: lysine ratios (110, 120, 130, 140, 150%) did not influence ($P>0.05$) the weight, percentage, and thickness of the shell (Table 3). Heat stress causes a negative impact on eggshell quality, however, even in conditions of thermal discomfort, no damage was observed to the eggshell quality characteristics, indicating that the calcium and phosphorus levels of the diets were adequate for the bird requirements. These characteristics are directly linked to specific gravity and SWUSA, which also did not obtain treatment effects ($P>0.05$).

Likewise, no significant effects of arginine: lysine ratios on weight, percentage, and yolk index were observed ($P>0.05$). The lack of effect of treatments on these parameters was expected since the yolk is the part of the egg that has the greatest capacity to retain lipids and is mainly influenced by the ingredients and the energy part of the diets.

The results obtained are similar to those found by Tuesta *et al.* (2018) evaluating five arginine: lysine ratios (101, 106, 111, 116 and 121%) in the laying Japanese quail diet, where there were no effects on productive performance and egg quality.

In another study, Bülbül *et al.* (2015) evaluated the effects of arginine supplementation (100 and 200 g/kg feed) with or without lysine supplementation (100 and 200 g/kg feed) in the diet of laying quails. As a result, the authors observed that supplementation of these amino acids did not affect egg production, egg weight or feed conversion ratio (per dozen eggs and per eggs mass), as well as Haugh unit, yolk index, albumen index and egg colour index yolk in terms of egg quality, being the control treatment, using the

Table 3. Egg quality traits of laying Japanese quails reared in hot climate as a function of digestible arginine: lysine ratios in the diet.

Variable	Arginine: lysine ratio (%)					SEM	p value ¹
	110	120	130	140	150		
Specific gravity (g/cm ³)	1.073±0.03	1.071±0.02	1.073±0.4	1.071±0.03	1.073±0.02	0.004	0.3314
Yolk weight (g)	3.191±0.37	3.222±0.15	3.163±0.12	3.192±0.15	3.21±0.15	0.026	0.9630
Albumen weight (g)	7.033±0.60	6.966±0.44	6.971±0.59	7.026±0.63	7.055±0.60	0.069	0.9930
Shell weight (g)	0.837±0.08	0.843±0.06	0.859±0.07	0.843±0.06	0.866±0.06	0.008	0.8279
%Yolk	28.48±1.38	29.37±0.95	29.09±1.11	29.63±1.32	29.69±0.98	0.156	0.0886
%Albumen	62.44±1.51	62.92±1.06	62.99±1.04	62.60±1.11	62.51±1.32	0.155	0.7372
%Shell	7.84±0.61	7.69±0.46	7.99±0.49	7.77±0.45	7.91±0.62	0.068	0.6730
Albumen height (mm)	4.969±0.39	4.862±0.67	4.985±0.51	4.857±0.60	4.877±0.51	0.068	0.9604
Yolk index	0.528±0.02	0.512±0.02	0.518±0.02	0.517±0.03	0.509±0.03	0.003	0.6206
Haugh unit	79.25±1.24	79.28±1.65	79.66±1.39	79.11±1.42	77.45±1.09	1.032	0.9702
Shell thickness (mm)	0.226±0.01	0.225±0.01	0.231±0.01	0.232±0.01	0.228±0.01	0.001	0.6107
SWUSA (mg/cm ²)	3.955±0.33	3.910±0.23	4.010±0.25	3.940±0.24	3.992±0.28	0.026	0.9007

SWUSA, shell weight per unit of surface area; SEM, Standard error of the mean. ¹Values >0.05 are considered non-significant.

arginine: lysine ratio of 126% recommended by the NRC (1994) sufficient to ensure a good productive performance of laying quails.

Therefore, the influence of the different ratios was not verified, suggesting the lowest dose studied (110%) as the ideal dose. However, despite being in accordance with recent studies that indicate lower doses than those recommended by Silva and Costa (2009) (132%) and Rostagno *et al.* (2017) (115%), the results obtained in this work support the hypothesis that the ratios presented in the literature are excessive. Hence, further dose-response studies with new arginine: lysine ratio arrangements are needed.

In conclusion, the 110% digestible arginine: lysine ratio, corresponding to 0.111 g/kg feed in the diet of laying Japanese quails reared in a hot climate, is sufficient to maintain the productive performance and the eggs quality.

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