Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2023 / v.25 / n.1 / 001-010

http://dx.doi.org/10.1590/1806-9061-2022-1653

Original Article

Adjusted Thr: Lys Ratio Improved the Performance and Efficiency of Japanese Quail

■Author(s)

Lima MR[I,IV,V] https://orcid.org/0000-0002-9897-6209
Costa FGP[II] https://orcid.org/0000-0003-4075-1792
Guerra RR[II] https://orcid.org/0000-0001-8226-8606
Vieira DVG[III] https://orcid.org/0000-0002-7407-9597
Cardoso AS[IV] https://orcid.org/0000-0002-8494-2032
Fernandes ML[V] https://orcid.org/0000-0001-7618-0798
Macena WG[IV] https://orcid.org/0000-0002-6225-3965
Nascimento DS[V] https://orcid.org/0000-0002-9688-4466
Rosendo HA[IV] https://orcid.org/0000-0003-3456-4675
Silva MRJ[IV] https://orcid.org/0000-0002-9098-2479
Oliveira JGR[IV] https://orcid.org/0000-0002-1364-5890
Santana AGS[V] https://orcid.org/0000-0001-9765-8981

[I] Animal Science, Federal Rural of Semi-Arid Region, Mossoró, RN, Brazil.
[II] Animal Science, Federal University of Paraiba, Areia, Paraiba, Brazil.
[III] Animal Science, Federal University of North of Tocantins, Araguaina, Tocantins, Brazil.
[IV] Federal University of South of Bahia, Itabuna, Brazil.
[V] Santa Cruz State University, Ilhéus, Bahia, Brazil.

■Mail Address

Corresponding author e-mail address
Matheus R. de Lima
Departamento de Ciências Animais. Av. Francisco Mota, 572 - Bairro Costa e Silva, Mossoró RN | CEP: 59.625-900.
Phone: 5584 992173987
Email: mrlmatheus@ufersa.edu.br

■Keywords

Quail nutrition, nutritional requirement, reproductive efficiency.

Submitted: 30/March/2022
Approved: 14/November/2022

ABSTRACT

Two hundred and forty Japanese quail aged 125 days were randomly allocated to five treatment groups based on laying (%) and quail's weight (90.71 ± 1.8% egg/day × 100 and 178.05 ± 9.38 g, respectively), each of which included six replicates of eight quails. The diets were formulated based on corn, soybean meal, and industrial amino acids. An optimal diet achieves 100% of amino acids required by the quail requirements, except for threonine. Five treatments were made: 20% less amino acid; 10% less amino acid; optimal diet; 10% more amino acid; and 20% more amino acids than those in the optimal diet. The increase in amino acid levels in a fixed Lys: amino acid ratio led to histological alterations in the liver and uterine epithelium, reduction in blood lipid peroxidation, lower hepatic HSP70 gene expression, and the performance of laying Japanese quail. The optimal diet based on the NRC with an adjusted Thr: Lys 78 ratio (Lys 1.0%) improved the performance and efficiency of Japanese quail from 125 to 230 days of age.

INTRODUCTION

An ideal relationship between amino acids must be maintained in diets because an imbalance in amino acids can negatively influence one's performance. The absorption of amino acids in imperfect balance can increase energy expenditure because of metabolism to eliminate excess nitrogen (Fernandes, 2012).

Lysine (Lys) is the second limiting amino acid in poultry feeds. Its basic need to correlate with other amino acids is fundamental for the best balance of amino acids, and the Lys level in a diet should be adjusted in terms of ideal protein levels. Their levels influence the quail performance (Mehri *et al.*, 2012) and quail protein metabolism (Wu, 1998). Thus, upgrading Lys levels in Japanese quail diets is necessary; however, it is not enough because this amino acid has a strong influence on others, so the relationship between them is important to be always considered.

The nutritional recommendations of the NRC (1994) for total amino acids are given, and over time there has been an improvement in quail, which may compromise the recommendation and use of these levels. However, it is still an important basis of use and dissemination among Japanese quail producers. Lima *et al.* (2013) commented that using inadequate levels of amino acids can induce a lower quail performance because amino acid deficiency limits protein synthesis when unused excess amino acids lead to more energy diverted to support nitrogen excretion.

The effect of threonine levels on the diet of Japanese quail was investigated by Lima *et al.* (2013), and it was concluded that threonine influenced the performance and histology of quail by improving their productive performance at a threonine: Lys ratio of 78%. Therefore,

it is necessary to improve this methodology to use amino acids in diets, with changes in amino acid: Lys ratio (Lima *et al.*, 2013) or dilution with no changes in amino acid: Lys ratio.

Tropical regions such as Brazil have temperature variations that put birds in a constant situation of heat stress, especially in the northeast of the country. In these cases, under stress conditions, the protective response of cells in the body of quail must be activated and it is through the gene expression of heat shock proteins, like HSP70, that it is initiated or increased (Li *et al.* 2021). This is because, according to Larkins *et al.* (2012), HSP proteins have a protective effect on tissues under stress, in order to maintain metabolism and structural integrity.

Therefore, this study was carried out to evaluate the effect of an increase in amino acid levels in diets, maintaining the amino acid: Lys ratio, on the performance, blood parameters, and histology of the liver, duodenum, magnum, and uterus of Japanese laying quail.

MATERIALS AND METHODS

Local, animals, ethical statement, and experimental period

The experiment was conducted in the Poultry Sector (Setor de Avicultura) of the Federal University of Paraíba (Universidade Federal da Paraíba - UFPB), Campus II, Areia, State of Paraíba, Brazil. All procedures performed in this study were approved by the Ethics and Research Committee of the Federal University of Paraíba, Areia, Brazil (approval number 37345/2013).

Two hundred and forty Japanese quail aged from 125 to 230 days (from the Fujikura® Company, Suzano, Sao Paulo, Brazil) were randomly allocated to five treatment groups based on laying (%) and quail's weight (90.71 ± 1.8% egg/day × 100 and 178.05 ± 9.38 g, respectively), each of which included six replicates of eight quail. The experimental period was divided into five periods of 21 days, totalizing 105 days. The quail were allocated in cages in clay-tile sheds with two water troughs. Eight quails were housed per cage (40 × 40 × 20 cm). The quail were exposed to a 17-h photoperiod throughout the experiment. The average temperature and relative humidity were 26.8 °C and 85%, respectively.

Diets and feeding program

The diets were formulated based on corn, soybean meal, and industrial amino acids (L-Lysine, DL-methionine, L-threonine, L-tryptophan, L-isoleucine, and L-valine). The levels of amino acids evaluated were based on Lys levels when diets were formulated by 0.80, 0.90, 1.00, 1.10, and 1.20 g/100 g, maintaining an optimal diet with 1.00 g/100 g Lys, as recommended by the NRC (1994). The other diets were based on NRC (1994) and Lima *et al.* (2013) to estimate and define the amino acids levels.

An optimal diet achieves 100% of amino acids required by the NRC (1994), except for threonine (Lima *et al.*, 2013). Five treatments were made: treatment 1: 20% less amino acid; treatment 2: 10% less amino acid; treatment 3: optimal diet; treatment 4: 10% more amino acid; and treatment 5: 20% more amino acids than those in the optimal diet.

All diets were formulated with free crude protein and fixed Lys: amino acid ratios based on the requirements by the NRC (1994), except for threonine (Lima *et al.*, 2013). The diets are shown in Table 1.

Data and sample collection

The following characteristics were studied: feed intake (g/quail/day), egg production (egg/day × 100), egg weight (g/egg), egg mass (g/egg), feed conversion per dozens of eggs (kg feed/dozen eggs), feed conversion per egg mass (kg feed/kg eggs), relative yolk weight (g/100 g of egg), relative albumen weight (g/100 g of egg), relative shell weight (g/100 g of egg), shell thickness (mm), and specific gravity (g/cm^3). This data was evaluated based cycle of 21 days. These data were collected and analyzed in each 21-day phases. Egg production data were recorded daily, calculated at the complete phase, and adjusted for mortality when there was. Feed intake was considered by the difference between offered and leftovers, adjusted for mortality when there was. Egg quality data in the last three days of each phase were analyzed.

At the end of the experimental period (230 days of age), the quail were euthanatized based CEUA approved, for optical microscopy, fragments of duodenum, liver, magnum, uterus, and kidneys from 10 quails for each treatment were included in paraplast and were subsequently sectioned into a series with 5-µm thickness. The histological staining methods used were hematoxylin and eosin, periodic acid-Schiff (PAS), and Masson's trichrome staining. Photomicrographs were captured using a micro-camera coupled to an Olympus BX-51 microscope (Olympus, Tokyo, Japan), and images were digitalized using a KS 400.3 software (Carl Zeiss Vision GmbH, Germany).

Table 1 – Ingredients and chemical composition of the experimental diets.

Items, g/100g	20% -AA	10% -AA	Optimal	10% +AA	20% +AA
Corn	72.003	69.184	68.525	67.866	67.208
Soybean meal	18.942	21.110	21.211	21.312	21.412
Limestone	5.445	5.439	5.438	5.437	5.435
Dicalcium phosphate	1.396	1.387	1.389	1.391	1.393
Soybean oil	0.611	0.980	0.951	0.923	0.895
Salt	0.521	0.521	0.522	0.522	0.522
L-Isoleucine	0.168	0.223	0.313	0.403	0.493
L-Lysine	0.168	0.218	0.317	0.416	0.515
L-Arginine	0.155	0.226	0.361	0.495	0.630
L-Threonine	0.131	0.183	0.261	0.339	0.417
L-Valine	0.118	0.179	0.273	0.367	0.461
DL-Methionine	0.116	0.171	0.242	0.313	0.384
L-Tryptophan	0.000	0.009	0.027	0.046	0.065
Choline	0.070	0.070	0.070	0.070	0.070
Vitamin mix[1]	0.050	0.050	0.050	0.050	0.050
Mineral mix[2]	0.050	0.050	0.050	0.050	0.050
Potassium carbonate	0.056	0.056	0.056	0.056	0.056
Calculated composition					
ME, MJ/kg	12.34	12.34	12.34	12.34	12.34
Crude protein, g/100g	15.091	16.060	16.412	16.763	17.114
Calcium, g/100g	2.500	2.500	2.500	2.500	2.500
Available phosphorus, g/100g	0.350	0.350	0.350	0.350	0.350
Sodium, g/100g	0.225	0.225	0.225	0.225	0.225
Chloride, g/100g	0.356	0.356	0.356	0.356	0.355
Potassium, g/100g	0.580	0.580	0.580	0.580	0.580
Total Lysine, g/100g	0.800	0.900	1.000	1.100	1.200
Total Methionine + cystine, g/100g	0.560	0.630	0.700	0.770	0.840
Total Tryptophan, g/100g	0.152	0.171	0.190	0.209	0.228
Total Valine, g/100g	0.736	0.828	0.920	1.012	1.104
Total Isoleucine, g/100g	0.720	0.810	0.900	0.990	1.080
Total Arginine, g/100g	1.008	1.134	1.260	1.386	1.512
Total Threonine, g/100g	0.623	0.701	0.779	0.857	0.935
Amino acid ratios by Lysine					
Met + Cys (NRC, 1994)	70	70	70	70	70
Trp (NRC, 1994)	19	19	19	19	19
Val (NRC, 1994)	92	92	92	92	92
Ile (NRC, 1994)	90	90	90	90	90
Arg (NRC, 1994)	126	126	126	126	126
Thr (Lima *et al.*, 2013)	78	78	78	78	78
Analyzed composition					
Crude protein, g/100g	15.1	16.2	16.7	16.8	17.5
ME, MJ/kg	12.28	12.31	12.30	12.35	12.31
Calcium, g/100g	2.5	2.5	2.5	2.5	2.5
Phosphorus, g/100g	0.3	0.3	0.3	0.3	0.3
Total Threonine, g/100g	0.62	0.71	0.78	0.86	0.94
Total Lysine, g/100g	0.80	0.90	1.00	1.10	1.20
Total Methionine, g/100g	0.46	0.51	0.63	0.72	0.76
Total Valine, g/100g	0.72	0.83	0.92	1.01	1.10

[1]Premix per kilogram of feed: vitamin A: 12,500 IU; vitamin D3: 3,000 IU; vitamin E: 150 mg; vitamin B1: 4 mg; vitamin B2: 8 mg; vitamin B6: 5 mg; vitamin B12: 0.030 mg; pantothenic acid: 20 mg; folic acid: 2.5 mg, vitamin K: 4 mg;

[2]Minimum premix per kilogram of feed: Mn: 56mg; Fe: 44 mg; Zn: 44 mg; Cu: 8.6 mg; I: 1 mg; Se: 0.34 mg.

Chemical analysis

To evaluate the dry matter content in the diets, samples were dried at 105 °C for 16 h (Aoac, 2006; method 934.01) in a drying oven. The N content was determined through a total combustion of the sample, while the Dumas method (Aoac, 2006; me-

thod 968.06) was used to calculate the CP content (N × 6.25). Ether extract (EE) was determined using the Soxhlet method (method 920.39), and the ash content was measured by burning the samples at 650 ºC overnight (method 942.05). The Ca and P contents were determined by Aoac International 2006 (method 984.01) and (method 965.17), respectively. A Parr adiabatic bomb calorimeter was used to determine the GE, using benzoic acid as an internal standard (Model 6200, Parr Instruments, Moline, IL).

The amino acid content of the diets was analyzed in triplicates in a commercial laboratory (Aoac, 1990) using method 982.30 E (a,b,c). Performic acid oxidation (Aoac, 1990) method 985.28 was conducted before acid hydrolysis for the determination of Met and Cys, whereas all other AA were determined after acid hydrolysis. Gross energy was determined using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL) and EE after a 3 N HCl acid hydrolysis (method Am 5–04) using an Ankom XT10 extraction system (Ankom Technology Corp. Macedon, NY), as described by Aocs and Mehlenbacher (2004). In addition, the diets were analyzed for P and Ca (method 985.01), as described by Aoac International (2000).

Lipid oxidation of blood

The lipid peroxidation in the blood from 10 quails for each treatment was determined by estimating malondialdehyde (MDA), following the methodology described by Draper and Hadley (1990), where 250 µL of serum was added in glass tubes, followed by 400 µL of 35% perchloric acid, and these were heated in a hot water bath for 1 h (37 ºC).

After 4h of fasting, at 9 am, the blood was obtained after bleeding in the euthanasia process using 50mL falcon tubes. After the fresh material is collected, the mixture was centrifuged (1400 g; 10 min); then, 600 µL of the supernatant was removed and added to 200 µL of thiobarbituric acid (1.2%). This mixture was heated in a hot water bath for 30 minº (95 ºC). Then, the solution was cooled, and reading was performed using a spectrophotometer (535 nm). A standard curve was obtained using 1,1,3,3-tetramethoxypropane, and the results obtained were expressed in nmol MDA/mL of serum.

HSP70 (mRNA level of HSP70)

Six pools of genomic DNA (10 birds/pool) were prepared for each studied treatment. The pools contained 6µg DNA and were digested with the restriction enzyme PstI (Gibco-BRL) for 8 h at 37 °C. The samples were then separated by electrophoresis in a 1% agarose gel for 6 h, at 50 V.

After electrophoresis, the gels were left in 0.2 N HCl for 10 min, washed rapidly using Milli-Q water and then immersed in a denaturing solution (0.5 N NaOH; 1.5 M NaCl) for 20 min. This procedure was done twice to assure DNA denaturation and the gels were washed once more with Milli-Q water and immersed in a neutralising solution (1 M Tris-HCl pH 7.5; 1.5 M NaCl) for 20 min. The DNA was transferred from the gel to nylon membranes (Immobilon- Ny+; SIGMA, N-8522) by Southern Blotting (Sambrook *et al.*, 1989).

After transference, the membranes were washed for 5 min with a wash solution (0.1 X SSC; 0.1% SDS; 50 mM Tris-HCl pH 7.5) and, shortly after, crosslinked at 160,000 µJ/cm2 to fix the DNA to the membrane. The membranes were placed in hybridisation bottles and 30 mL of pre-hybridisation solution (6X SSC; 10X Denhardt's reagent; 1% SDS; 250 µg denatured salmon sperm DNA/mL) was added. The bottles were placed in a hybridisation oven (HYBAID Instruments) at 65 °C for 2 h. The pre-hybridisation solution was discarded and 15 mL of pre-warmed hybridisation solution (6X SSC; 1% SDS, 65 °C) was added with 10 µL of probe labelled with 32P-dCTP by random priming (Feinberg & Volgestein, 1983).

The probe was a DNA fragment specific for the chicken hsp70 gene, amplified using the primers Hsp70-F1 and Hsp70-R3. The fragment length was 554 bp and it comprised both the promoter and the beginning of the coding region of hsp70 gene. After 16 h of hybridisation, the solution was changed to 50 mL of washing solution 1 (2X SSC, 0.1% SDS), pre-warmed to 65 °C. The membranes were washed twice for 20 min using washing solution 2 (0.1X SSC; 0.1% SDS, 65 °C). Sheets of X-Ray film were exposed to the membranes using adequate cassettes for 72 h at -70 °C and developed.

The mRNA levels of HSP70 were measured by reverse transcription (RT)-PCR. The total RNA was isolated from the frozen in nitrogen tissues using a TRIzol reagent (Life Technologies Co., Ltd., Carlsbad, CA), following the manufacturer's protocols. Primers were designed using a Primer Express software (Applied Biosystems). The quality and integrity of the total RNA were evaluated using a NanoDrop spectrophotometer (Thermo Scientific). Reverse transcription was carried out using a Prime ScriptTM RT reagent kit (TaKaRa) with random hexamers, as recommended by the manufacturer.

HSP70 in liver

F1: 5'GAGTGGCGCAGCGTAGAAAG 3', poisition on reference sequence: 18

R3: 5'CACTTGGTTCTTGGCAGCATC 3', poisition on reference sequence: 571

Seric levels

The seric levels of total proteins, albumin, and uric acid were evaluated using semi-automated bioanalysis (BioPlus® BIO-2000) and commercial kits. The seric globulin levels were calculated using the difference between the seric levels of total proteins and albumin.

Statistical analysis

Statistical analysis of the characteristics studied was performed using the Statistical Analysis Software (SAS User's Guide 2004). The assumptions of the analysis of variance (error normality, random and independent errors, and variance homoscedasticity) were met. The recommendations were obtained by polynomial regressions.

RESULTS

Performance analysis

Feed intake (Table 2) was affected linearly (p=0.004) by the amino acid levels in the diet. However, egg production (p<0.001), egg mass (p<0.001), and feed conversion to egg mass (p<0.001) and to dozens of eggs (p<0.001) were influenced in a quadratic manner. The equations and estimates of the requirements are shown in Table 3.

Egg production was increased until it reached the level of 10% of increase in amino acids in the diet, followed by a drop in productivity after this level. There was an increase in responses to egg mass (p<0.001) and feed conversion to egg mass (p<0.001) and dozen eggs (p<0.001), as shown in Table 2.

Table 2 – Feed intake (FI, g/quail/day), egg production (EP, egg/day ×100), egg weight (EW, g/egg), egg mass (EM, g/egg), feed conversion per egg mass (FCEM, kg feed/kg eggs), and feed conversion per dozens of eggs (FCDE, kg feed/dozen of eggs) according to the experimental diets.

Diet	FI	EP	EW	EM	FCEM	FCDE
20% - AA	24.71	76.13	11.19	8.51	2.91	0.39
10% - AA	25.12	79.47	11.33	9.01	2.80	0.38
Optimal	25.47	82.63	11.66	9.63	2.65	0.37
10% + AA	25.30	81.96	11.67	9.57	2.65	0.37
20% + AA	26.02	76.44	11.51	8.80	2.97	0.41
p-value						
Linear	0.004	0.339	0.070	0.061	0.872	0.108
Quadratic	0.931	<0.001	0.148	<0.001	<0.001	<0.001
Lack of fit	0.421	0.336	0.708	0.331	0.177	0.119
SEM†	0.279	1.004	0.1658	0.1848	0.0591	0.0054

†Standard error means.

Table 3 – Equations and estimated recommendations per variable by Lys level.

Variable	Equation	R^2	BreakPoint
Feed intake	FI = 2.8Lys + 22.524	0.85	-
Egg production	Ep=-153.93Lys^2 + 310.97Lys – 74.634	0.94	1.01
Egg mass	EM = -23Lys^2 + 47.14Lys– 14.576	0.92	1.02
FCR egg mass	FCEM = 7.2143Lys^2– 14.459Lys + 9.896	0.85	1.00
FCR dozen of eggs	FCDE = 0.7857Lys^2– 1.5414Lys + 1.124	0.85	0.98

Feed intake (FI, g/quail/day), egg production (EP, egg/day x100), egg mass (EM, g/egg), feed conversion per egg mass (FCEM, kg feed/kg eggs) and feed conversion per dozens of eggs (FCDE, kg feed/ dozen of eggs).

Egg quality analysis

Based on the levels of amino acids present in the studied diets (Table 4), the treatments had no significant effect on the variables related to egg quality: specific specification (p>0.05), Haugh unit (p>0.05), relative albumin weight (p>0.05), shell (p>0.05), and yolk (p>0.05).

Optical microscopy analysis

As for the histological structure, the uterus of quail between the groups showed a difference when the amino acid levels were reduced by 20%. There was no histological difference in the magnum and duodenum among the studied treatments (data not shown).

Table 4 – Specific gravity (SG, g/cm^3), Haugh unit (HU), and relative weight of albumen (RAL, g/100g of egg), shell (RSW, g/100g of egg), and yolk (RYW, g/100g of egg) of the eggs in the experimental diets.

Diet	SG	HU	RAL	RSW	RYW
20% - AA	1.071	91.96	52.92	15.20	31.88
10% - AA	1.071	91.79	53.23	15.16	31.61
Optimal	1.070	93.30	53.06	14.91	32.03
10% + AA	1.070	91.21	52.57	15.33	32.10
20% + AA	1.070	91.19	52.81	15.44	31.75
p-value					
Linear	0.145	0.411	0.598	0.678	0.891
Quadratic	0.258	0.281	0.816	0.594	0.792
Lack of fit	0.840	0.279	0.754	0.897	0.788
SEM†	0.0004	0.8037	0.5196	0.4862	0.5288

†Standard error mean.

In all treatments, the liver appeared to be steatotic, with lipids found mainly around the hepatic triads and with no parenchymal collagen deposition. However, in the liver of quail in the optimal diet treatment, a significantly higher weight (Figure 2) and more advanced steatoses were observed (Figure 1A). There were also less glycogen storage (Figure 1C) and thicker uterine epithelium folds (Figure 1E) in comparison to those in the treatment with 20% reduction in amino acids levels (Figure 1B, Figure 1C, and Figure 1F).

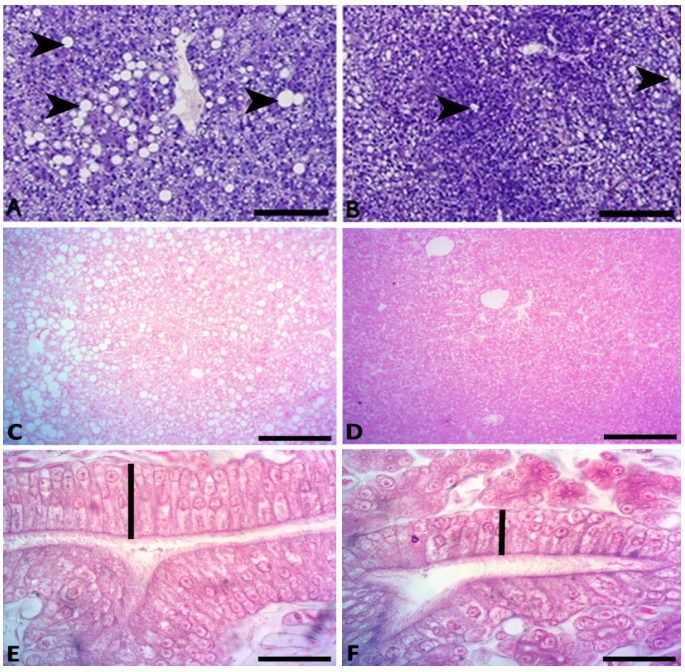

Figure 1 – Photomicrographs of the liver and uterus of laying Japanese quail to qualitative comparison. A) Individual from the Optimal diet treatment (with 1.0g/100g lysine), showing severe steatosis in the liver characterized by droplets of lipid (arrowhead). B) Individual representative of the 20% of reduction of amino acid levels treatment with moderate steatosis in the liver. A, B) Hematoxylin-eosin stain. C) Individual from the Optimal diet treatment showing lesser glycogen storage (lesser positivity for Periodic Acid Schiff-PAS). D) Individual representative of the 20% of reduction of amino acid levels treatment with more glycogen storage. C, D) PAS stain. E) Individual from the Optimal diet treatment showing thicker uterine mucosa folds (vertical bar). F) Individual representative of the 20% of reduction of amino acid levels treatment showing thinner uterine mucosa folds (vertical bar = height of the epitelium). E, F) Trichome's Masson stain. A, B, C, D) Bar: 400 µm. E, F) Bar: 80 µm.

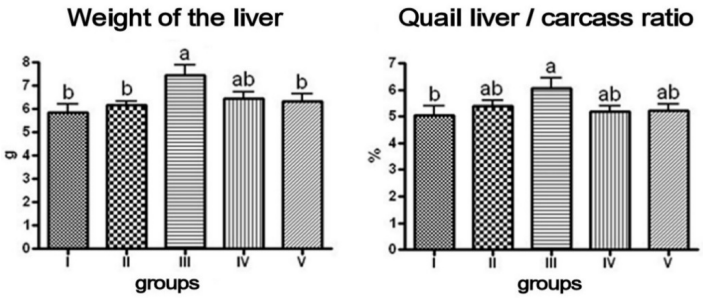

Figure 2 – Weight of the liver and liver/carcass ratio in the different treatments for laying Japanese quail at 230 d of age, 10 quail per treatment at 230 d of age. Treatment I: 20% less amino acid by Optimal diet; Treatment II: 10% less amino acid by Optimal diet; Treatment III: Optimal diet; Treatment IV: 10% more amino acid by Optimal diet; and Treatment V: 20% more amino acid by Optimal diet. ANOVA, test Tukey ($p \leq 0.05$).

Seric level analysis

The blood variables evaluated in the study are shown in Table 5. There was a decreasing linear effect on the levels of MDA (p=0.002). The total protein levels did not have a significant effect on the levels of Lys studied.

Albumin levels were increased linearly by the diets (p=0.017), while globulin levels were not. Additionally, blood uric acid levels were decreased linearly (p<0.001), which was a good sign.

The HSP70 gene expression also showed a linear response (p<0.001), and it seems that the increase in the levels of Lys and other amino acids maintaining the relationship is more important than the isolated levels of amino acids.

Weight of the liver and liver/carcass ratio

The weight of the liver and liver/carcass ratio was obtained at 230 d of age of Japanese quail. These data were compared by ANOVA, test Tukey ($p \leq 0.05$) to permit to determine the difference about nutritional program.

Table 5 – MDA (nmol MDA/mL), total protein (PTN, g/L), albumin (ALB, g/L), globulin (GLOB, g/L), uric acid (UA, mmol/L), and gene expression of HSP70 (HPP70, Alleles) gene in the blood of quail fed by crescent amino acid in levels.

Diet	MDA, Blood	PTNT	ALB	GLOB	UA	HSP70
20% - AA	4.483	5.368	2.081	3.420	52.106	1.184
10% - AA	4.392	5.443	2.244	3.483	52.481	0.996
Optimal	4.236	5.168	2.206	3.408	50.374	0.917
10% + AA	4.125	5.280	2.306	3.308	49.388	0.864
20% + AA	4.179	5.318	2.331	3.358	51.106	0.872
p value	0.006	0.417	0.037	0.722	0.098	<0.001
Linear	0.002	0.433	0.017	0.424	0.045	<0.001
Quadratic	0.452	0.493	0.538	0.649	0.758	<0.001
lack of fit	0.585	0.147	0.218	0.655	0.175	0.027
SEM	0.073	0.1113	0.0599	0.1031	0.7957	0.009
CV	5.14	6.33	8.11	9.19	4.71	2.84
Variable	Effect		Equation		r^2	
MDA, Blood	Linear		Y= -0.842753Thr/Lys + 5.137576		93	
ALB	Linear		Y=0.507143Thr/Lys + 1.716786		76.21	
UA	Linear		Y=-5.59632Thr/Lys + 56.75838		68.32	
HSP70	Linear		Y= -0.72763Thr/Lys + 1.700637		80.16	

DISCUSSION

The result of feed intake (FI) differs from those found by Ribeiro *et al.* (2013), who worked with levels of Lys (0.95, 1.00, 1.05, 1.10, 1.15, and 1.20%), which did not have a statistical effect for FI. When evaluating five levels of Lys (0.88, 0.96, 1.04, 1.12, and 1.20%), Costa *et al.* (2008) observed a decreasing linear effect. When evaluating the levels of 8.80, 9.60, 10.40, 11.20, and 12.00 g of Lys/kg of feed for Japanese quail in the laying phase, Rodrigues *et al.* (2007) observed a quadratic effect. The increase in feed intake due to the Lys levels in the diets possibly occurred due to the low level of crude proteins that did not follow the recommendations of the NRC (1994), as quail tend to regulate food consumption according to their nutritional needs.

Egg production was increased according to Leclercq (1998). The excess amino acids ingested by birds are catabolized and excreted in the form of uric acid. Moreover, according to Costa *et al.* (2001), a metabolic expenditure of 6–18 mol of ATP, which may vary according to the amount of nitrogen from the ingested amino acid, is lost by the birds in the catabolism process. Therefore, the optimal use of 10% more Lys and all amino acids would maintain a greater amount of energy for egg production, even keeping the crude protein levels below those established by the NRC (1994), with an egg production level >82%.

The increase in responses to egg mass and feed conversion to egg mass and in a dozen eggs (p<0.001) is in line with that found by Mauricio *et al.* (2018), who estimated a level of 1.12 g of Lys/100 g of diet and with the results obtained by Pinto *et al.* (2003), where 1.11 g of Lys/100 g of feed was what maximized the egg mass. Demuner *et al.* (2009) also found a positive influence of the level of Lys on the mass feed conversion of laying Japanese quail, with the best responses obtained at the level of 1.09%.

The results for the conversion by a dozen eggs differed from those observed by Mauricio *et al.* (2018) and Demuner *et al.* (2009), who did not observe a statistical effect on the researched Lys levels. Ribeiro *et al.* (2013) evaluated the requirement for Lys (0.95, 1.00, 1.05, 1.10, 1.15, and 1.20%) for laying Japanese quail and concluded that to provide a good performance and egg quality, quail diets must contain 1.12% Lys. This differences in results may be linked to the fact that the feed intake has suffered a statistical difference in the present study, explaining the results obtained for the feed conversion per dozen eggs.

The studies above were concerned with Lys levels only. Therefore, doses of isolated amino acids in the diet were assessed, as they may indirectly alter the relationship between amino acids. In this study, in addition to the modification of amino acid levels, the amino acid: Lys ratio was maintained, so the result cannot be compared only in terms of the Lys levels, or another amino acid evaluated.

However, the data listed above show a surprising similarity between the two forms of use of amino acids in the diet of quail. The first and the most common was when the supplementation was based on the isolated amino acid level, with changes in the amino acid: Lys ratio, while the second, according to the data presented in this study, had a supplementation

of several amino acids, keeping the relationship fixed. Thus, based on the assessment of the need for amino acids for Japanese quail, it can be stated that these have probably no influence, whether they maintain the amino acid: Lys ratio.

For the histological structure, the uterus of quail between the groups showed a difference. A 20% reduction in amino acids in the diets resulted in thin uterine epithelium folds (Figure 1F). This indicates that low amounts of amino acids in the diet for laying Japanese quail may lead to a less efficient shell production. Such characteristics permit shell formation for a long time and consequently decrease egg production, since the egg stays in the uterus for variable times for the deposition of calcium carbonate (King & Mclelland, 1979).

All treatments the liver appeared to be steatotic, and the increase in the liver weight may be explained by the higher deposition of fat, with a lipid found in hepatic triads, but without parenchymal collagen deposition, so, this results may be due to the high estrogen synthesis in the ovary to support the greater egg production (Bunchasak & Silapasorn, 2005) among the evaluated treatments, as shown in Table 2.

Another effect that may explain the greater egg production in quail fed with the optimal diet or diets with increased amino acid levels is the little positivity of the liver to PAS staining (Figure 1C, D). This result indicates the little deposition of hepatic glycogen, demonstrating a greater destination of this source of energy to other organs. In this case, its major destination would be toward the reproductive system, which would improve the capacity for egg production (Figure 1, E). This is also because the quail fed with the optimal diet were efficient in daily egg production (82.63 egg/day × 100/quail) compared to those in the other treatments.

It is important to mention that despite the increase in liver steatosis, there were no depots of liver parenchymal collagen in the quail fed with the optimal diet, which would denote a fibrotic process due to a continuous hepatocyte regeneration (Fausto, 2003). The liver/carcass ratio was also higher in quail fed with the optimal diet (Figure 2).

The quail fed with the optimal diet had histological traits that propitiate quail-performance rates of greater efficiency in relation to the other treatments. This was based on the histological evaluations, which were validated by the results observed in the Japanese quail' performance data, as shown in Table 2.

MDA can represent the lesion from tissue lipid peroxidation, and it directly reveals the severity of the attack by free radicals (Wu *et al.*, 2007; Khan *et al.*, 2015). We can infer that the increase in the levels of Lys and other amino acids, maintaining a constant ratio between them, is beneficial in terms of lipid oxidation.

There was no effect on the total protein. This indicates the absence of problems, especially with the reduction of this variable. This can indicate cases of nephropathy and chronic liver diseases, inadequate nutrition, absorptive deficiency, neoplasms, and chronic blood loss (Rupley, 1999) or excessive hydration (Campbel *et al.*, 2014). High levels can be observed in cases of dehydration or chronic infectious diseases that increase the production of globulins (Rupley, 1999).

According to dos Santos *et al.* (2007), plasma serum albumin concentrations tend to be affected during the productive period of birds, since albumin is a precursor protein to the yolk, synthesized in the liver, and carried to the ovary, where it is transferred to the oocytes. Albumin is used as an indicator of liver function, and its synthesis is a characteristic function of healthy liver cells (Noureddin & Loomba, 2012; Mikolasevic *et al.*, 2014).

A low albumin level, which was associated with a high globulin level, may be an indicator of liver dysfunction. The changes in uric acid levels in the blood serum are associated with nitrogen metabolism in response to the levels of amino acids present in the diets that may be adequate or deficient (Kohn *et al.*, 2005; Donsbough *et al.*, 2010), and a reduction in uric acid levels is an indicator of a better use of the protein provided (Donsbough *et al.*, 2010); this was observed in the treatment at an optimal level and with a 10% increase in amino acid levels.

Expression of heat shock proteins (Hsp) is induced in all cells by exposure to heat and other environmental stresses, and Hsp can protect cells from damage through further exposure. The expression of the HSP70 gene due to the levels of Lys and other amino acids must consider the benefit generated for performance, egg quality, greater antioxidant capacity and stress tolerance, in addition to better health and efficiency of the liver and reproductive tract. In this study, the expression was increased in the diet with the lowest level of amino acids, and the opposite in the diet with increased levels of these nutrients. Thus, we can infer that an amino acid adjusted diet such as this study increases heat tolerance capacity, because it has a lower hepatic hsp70 gene expression.

Based on this, in conclusion, the increase in amino acid levels in a fixed Lys: amino acid ratio led to reduction in lipid peroxidation, lower HSP70 gene expression, and promotes a histological alteration in the liver and uterine mucosa that increased egg production capacity, which the performance of laying Japanese quail is improved. The optimal diet based on the NRC with an adjusted Thr: Lys 78 ratio (Lys 1.0%) improved the physiological changes that allows to increase performance and efficiency of Japanese quail from 125 to 230 days of age.

STATEMENT OF ANIMAL RIGHTS

The experiment was submitted for approval by the Comissão de Ética no Uso de Animais (Animal Research Ethics Committee, CEUA, 37345/2013) of the Federal University of Paraíba (UFPB), Brazil.

CONFLICT OF INTEREST STATEMENT

The authors confirm that there are no known conflicts of interest associated with this publication.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and CONFEA (Conselho Federal de Uso e Experimentação Animal, Brazil).

REFERENCES

AOCS - American Oil Chemists' SocietyC. Official methods and recommended practices of the AOCS. Illinois; 2004.

AOAC. - Association Of Official Analytical Chemists. Official methods of analysis of AOAC International. 17th ed. Gaithersburg; 2000.

AOAC. Association Of Official Analytical Chemists. Official Methods Of Analysis Of Aoac Internationalmethods of analysis of AOAC international. 15th ed. Journal of Association of Analysis ChemicalsWashington; 1990.

Bunchasak C, Silapasorn T. Effects of adding methionine in low-protein diet on production performance, reproductive organs and chemical liver composition of laying hens under tropical conditions. International Journal of Poultry Science 2005;5:301-8.

Campbel TW. Bioquímica Clínica das aves. In: Thrall MA, Weiser G, Allison RW, Campbell TW. Hematologia e bioquímica Clínica Veterinária. 2Nd ed. São Paulo: Roca; 2014. p.688.

Costa FGP, Rostagno HS, Albino LFT, Gomes PC, Toledo RS, Vargas Junior JG. Níveis dietéticos de proteína bruta para frangos de corte de 1 a 21 e 22 a 42 dias de idade. Revista Brasileira de Zootecnia 2001;30(5):1498-505.

Costa, FGP, Rodrigues VP, Goulart CC, Lima Neto RC, Souza JGD, Silva JHV. Exigências de lisina digestível para codornas japonesas na fase de postura. Revista Brasileira de Zootecnia 2008;37(12):2136-40.

Demuner LF, Vargas JG, Scottá BA. Níveis nutricionais de lisina digestível para codornas japonesas alimentadas com rações contendo 19,5% de proteína bruta. Anais da 5th ZOOTEC; 2009; Águas de Lindóia, São Paulo. Brasília: Associação Brasileira de Zootecnia; 2009.

Donsbough AL, Powell S, Waguespack A, Bidner TD, Southern LL. Uric acid, urea, and ammonia concentrations in serum and uric acid concentration in excreta as indicators of amino acid utilization in diets for broilers. Poultry Science 2010;89(2):287-94.

Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods in Enzymology 1990;186:421-31.

Fausto N. New perspectives on liver regeneration. Hepatology 2003;6(2):326-7.

Feinberg AP, Volgestein BA. A technique for radio- labeling DNA restriction endonuclease fragments to high specific activity. Analytical Biochemistry 1983;132:6-13.

Fernandes MNS. Aminoácidos digestíveis na nutrição de frangos de corte. Revista Eletrônica Nutritime 2012;9(6):2135-53.

Khan RA, Khan MR, Sahreen S, Ahmed M, Shah NA. Carbon tetrachloride-induced lipid peroxidation and hyperglycemia in rat: a novel study. Toxicolology Industry Health 2015;31(6):546-53.

King AS, McLelland J. Form and function in birds. London: Academic Press; 1979.

Kohn RA, Dinneen MM, Russek-Cohen E. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. Journal of Animal Science 2005;83(4):879-89.

Leclercq, B. Lysine: specific effects of lysine on broiler production: comparison with threonine and valine. Poultry Science 1998;77(1):118-23.

Li RZ, Wei H, Wang Y, Chen Y, Zhang H, Li X, *et al.* Enriched environment housing improved the laying hen's resistance to transport stress via modulating the heat shock protective response and inflammation, Poultry Science 2021;100(3).

Lima MR, Costa FGP, Guerra RR, Silva JHV, Rabello CBV, Miglino MA, *et al.* Threonine: lysine ratio for Japanese quail hen diets. The Journal of Applied Poultry Research 2013;22(2):260-8.

Maurício TV, Vargas Junior JG, Souza MF, Costa BF, Nunes RV, Vieites FM. Ratio of digestible lysine to arginine in Japanese laying quails. Semina Ciências Agrárias 2018;39(1):299-310.

Mehri M, Davarpanah AA, Mirzaei HR. Estimation of ideal ratios of methionine and threonine to lysine in starting broiler chicks using response surface methodology. Poultry Science 2012;91(3):771-7.

Mikolasevic I, Lukenda V, Racki S, Milic S, Sladoje-Martinovic B, Orlic L. Nonalcoholic fatty liver disease (NAFLD)–a new factor that interplays between inflammation, malnutrition, and atherosclerosis in elderly hemodialysis patients. Clinical Intervencion Aging 2014;9:1295-303.

Noureddin M, Loomba R. Nonalcoholic fatty liver disease: Indications for liver biopsy and noninvasive biomarkers. Clinical Liver Disease 2012;1(4):104.

NRC – National Research Council. Nutrient requirements of poultry. 9th ed. Washington: National Academies Press; 1994.

Pinto R, Donzele JL, Ferreira AS, Albino LFT, Soares RDTRN, Silva MDA, *et al.* Exigência de metionina mais cistina para codornas japonesas em postura. Revista Brasileira de Zootecnia 2003;32(5):1166-73.

Ribeiro CLN, Barreto SLDT, Reis RDS, Muniz JCL, Donzele JL, Gomes PC, *et al.* Digestible lysine levels in diets for laying Japanese quails. Revista Brasileira de Zootecnia 2013;42(7):489-95.

Rodrigues VP, Costa FGP, Silva JHV. Exigência de lisina para codornas japonesas em produção. Anais do Simpósio Internacional de Coturnicultura; 2007; Lavras, MG: UFLA; 2007. p.173.

Rupley AE. Manual de clínica aviária. São Paulo: Editora Roca; 1999.

Sambrook J, Fritsch EF, Maniatis T. Molecular Clon- ing: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Press; 1989.

SAS User's guide. Version 9.1 ed. Cary: SAS Institute; 2004.

Schmidt EMS, Paulillo AC, Santin E, Dittrich RL, Oliveira EG. Hematological and serum chemistry values for the ring-necked pheasant (Phasianus colchicus): variation with sex and age. International Journal of Poultry Science 2007;6:137-9.

Wu G. Intestinal mucosal amino acid catabolism. Journal of Nutrition 1998;128:1249-52.

Wu Y, Li L, Wen T, Li YQ. Protective effects of echinacoside on carbon tetrachloride induced hepatotoxicity in rats. Toxicology 2007;232(1-2):50-6.