

**Segundo aminoácido limitante em dietas à de milho e farelo de soja para codornas  
japonesas**

**Second-limiting amino acid in corn-soybean meal based diets for Japanese quail**

**Segundo aminoácido limitante en las dietas de harina de maíz y soya para codornices  
japonesas**

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**Resumo**

Objetivou-se determinar o segundo aminoácido limitante em dietas à base de milho e farelo de soja para codornas japonesas nas fases de crescimento e de postura. Um total de 1.440 codornas japonesas foi utilizado em três experimentos nas fases de 1 a 21; 22 a 42 e 85 a 210 dias de idade, respectivamente. Cada experimento continha oito tratamentos com seis repetições de dez aves. As dietas experimentais foram baseadas em milho e farelo de soja. Os níveis de metionina + cisteína foram adequados em todas as dietas experimentais, porém houve variação no atendimento de mais um segundo aminoácido essencial. Nos experimentos 1 e 2, as codornas que foram alimentadas com dietas com níveis adequados de metionina + cisteína e lisina apresentaram maior ganho de peso (g/ave) e melhor conversão alimentar (g/g). O consumo de ração (g/ave) não foi influenciado. No experimento 3, os níveis adequados de todos os aminoácidos promoveram uma menor ingestão de ração (g/ave) e melhor conversão alimentar por massa de ovos (g/g) e por dúzia de ovos (g/dúzia). O peso do

ovo (g), o peso (g) e a espessura da casca (mm) e a gravidade específica ( $\text{g}/\text{cm}^3$ ) não foram afetados. As dietas que atenderam aos requisitos de todos os aminoácidos promoveram a postura de ovos com maior peso de albúmen (g) e menores pesos de gema (g) de ovo. Em conclusão, o segundo aminoácido limitante das dietas vegetais para codornas japonesas é a lisina.

**Palavras-chave:** *Coturnix coturnix japônica*; Desempenho zootécnico; Lisina; Nutrição aminoacídica; Produção de ovos; Qualidade dos ovos.

### Abstract

The objective was to determine the second-limiting amino acid in diets based on corn-soybean meal for Japanese quails in the growth and laying stages. A total of 1,440 Japanese quails were used in three experiments in phases 1 to 21; 22 to 42 and 85 to 210 days of age, respectively. Each experiment contained eight treatments with six replications of ten birds. The experimental diets were based on corn-soybean meal. The levels of methionine + cysteine were adequate in all experimental diets, but there was variation in the attendance of another second essential amino acid. In experiments 1 and 2, quails that were fed diets with adequate levels of methionine + cysteine and lysine showed higher weight gain ( $\text{g}/\text{bird}$ ) and better feed conversion ( $\text{g}/\text{g}$ ). Feed intake ( $\text{g}/\text{bird}$ ) was not influenced. In experiment 3, adequate levels of all amino acids promoted a lower feed intake ( $\text{g}/\text{bird}$ ) and better feed conversion per egg mass ( $\text{g}/\text{g}$ ) and per dozen eggs ( $\text{g}/\text{dozen}$ ). Egg weight (g), weight (g) and shell thickness (mm) and specific gravity ( $\text{g}/\text{cm}^3$ ) were not affected. The diets that met the requirements of all amino acids promoted the laying of eggs with a greater weight of albumen (g) and lower weights of egg yolk (g). In conclusion, the second-limiting amino acid in plant diets for Japanese quails is lysine.

**Key words:** *Coturnix coturnix japonica*; Growth performance; Lysine; Amino acid nutrition Egg production; Egg quality.

### Resume

El objetivo fue determinar el segundo aminoácido limitante en dietas a base de harina de maíz y soja para codornices japonesas en etapa de crecimiento y puesta. Se utilizó un total de 1.440 codornices japonesas en tres experimentos en las fases 1 a 21; 22 a 42 y 85 a 210 días de edad, respectivamente. Cada experimento contenía ocho tratamientos con seis repeticiones de diez aves. Las dietas experimentales se basaron en harina de maíz y soja. Los niveles de metionina + cisteína fueron adecuados en todas las dietas experimentales, pero hubo variación

en la presencia de otro segundo aminoácido esencial. En los experimentos 1 y 2, las codornices que fueron alimentadas con dietas con niveles adecuados de metionina + cisteína y lisina mostraron un mayor aumento de peso (g/ave) y una mejor conversión alimenticia (g/g). La ingesta de alimento (g/ave) no se vio afectada. En el experimento 3, los niveles adecuados de todos los aminoácidos promovieron una menor ingesta de alimento (g/ave) y una mejor conversión alimenticia por masa de huevo (g/g) y por docena de huevos (g/docena). El peso del huevo (g), el peso (g) y el grosor de la cáscara (mm) y la gravedad específica ( $\text{g/cm}^3$ ) no se vieron afectados. Las dietas que cumplieron con los requisitos de todos los aminoácidos promovieron la puesta de huevos con un mayor peso de albúmina (g) y menor peso de yema de huevo (g). En conclusión, el segundo aminoácido limitante en las dietas vegetales para las codornices japonesas es la lisina.

**Palabras clave:** *Coturnix japonés coturnix*; Rendimiento zootécnico; Lisina; Nutrición de aminoácidos; Producción de huevos; Calidad del huevo.

## 1. Introduction

The development of quail production has been closely accompanied by technological advances in facilities, equipment and expertise in the areas of management and nutrition; however, some basic nutrition and diet formulation concepts have not been elucidated. Vieira, et al. (2020) show that more research on Japanese quail nutrition must be carried out to clarify the requirements for metabolizable energy. One of the most studied nutritional strategies is the reduction of crude protein (CP) combined with crystalline amino acids (Alagawany, et al., 2014), which decreases feeding costs and mitigates ammonia emissions.

The diet must provide the animal with necessary nutrients for maintenance, as well as those needed for egg laying (Lima, et al., 2020). Protein is one of the most important nutrients for bird development; however, animals require nutrients in the forms of individual essential or non-essential amino acids that are not present in the CP itself (Lima, et al., 2014). Methionine (Met) is responsible for initiating the synthesis of the polypeptide chain and serves as a methyl donor group for various metabolic reactions (Warnick & Anderson, 1968), and proved indispensable for the maximum performance of quails feed with diets low-protein (Saraiva, et al., 2020).

Lysine (Lys) is related to protein synthesis and deposition, and participates in the synthesis of carnitine, which plays a role in the transport of fatty acids for mitochondrial  $\beta$ -oxidation (Costa, et al., 2001). Hajkhodadadi, et al. (2014) conducted an experiment with 21-

to 42-day-old Japanese quail and found that 1.45% digestible Lys was sufficient to promote the maximum weight gain. Mehri, et al. (2015) suggested that the ideal Lys level to optimize the growth (7- 21) of Japanese quail was 1.34%, and for 21-35 days-old, the ideal Lys level is 1.36% of the diet. Lima, et al. (2016) suggested that the ideal Lys level to optimize the growth (1-40 days) of Japanese quail was 1.20%. Hasanvand, et al. (2017) observed that levels below 1.24% Lys, limited the development of quails.

Each amino acid plays an important role in animal development. For this reason, minimal concentrations should be provided in the diet; when these concentrations are not met, the animal performance is limited. In this regard, meeting the minimum requirement of each amino acid follows a limiting order, which is affected by different factors such as the age of the animal, physiological functions and the basic ingredients used in the feed formulation.

In corn-soybean meal-based diets, Met and Lys are the first- and second-limiting amino acids, respectively, for broiler chickens and laying hens. For Japanese quail, Met has been confirmed as the first-limiting amino acid (Mandal, et al., 2005; Parvin, et al., 2009), but information about the next-limiting amino acid not are known. Therefore, the present study aimed to determine the second-limiting amino acid in corn-soybean meal based diets for Japanese quail during the growth (1-42 days) and egg laying phases.

## **2. Materials and Methods**

### ***Ethics committee approval, birds, and dietary treatments***

The research was conducted in the Poultry Science Sector of the Agrarian Sciences Center of Federal University of Paraiba, localized in Areia-PB. All procedures were approved by the Animal Ethics Committee this institution.

A total of 1,440 Japanese quails were used in three experiments in phases 1 to 21; 22 to 42 and 85 to 210 days of age, respectively. Each experiment contained 8 treatments with six replications and 10 birds per replication, according to a completely randomized design.

The diets were formulated following specific recommendations (Silva & Costa, 2009). The control treatment was a diet with adequate levels of Met formulated to meet the methionine and cysteine (Met+Cys) requirements; all other experimental diets has the control treatment plus one second essential amino acid, and furthermore others essential amino acids was below off requirements for limited the performance. In the Table 1 shows the treatment

designer. The percentage composition of the experimental diets are presented in the Tables 2, 3, and 4.

**Table 1.** Treatment design.

Requirements met	Requirements met	Below requirements
1- Met+Cys <sup>A</sup>	Met+Cys	Lys, Thr, Trp, Val, Arg and
2- Met+Cys and Lys	Met+Cys and Lys	Thr, Trp, Val, Arg and Ile
3- Met+Cys and Thr	Met+Cys and Thr	Lys, Trp, Val, Arg and Ile
4- Met+Cys and Trp	Met+Cys and Trp	Lys, Thr, Val, Arg and Ile
5- Met+Cys and Val	Met+Cys and Val	Lys, Thr, Trp, Arg and Ile
6- Met+Cys and Arg	Met+Cys and Arg	Lys, Thr, Trp, Val and Ile
7- Met+Cys and Ile	Met+Cys and Ile	Lys, Thr, Trp, Val and Arg
8- All <sup>B</sup>	Met+Cys, Lys, Thr, Trp, Val, Arg	--

<sup>A</sup>Control diet = diet with an adequate level of methionine formulated to meet the requirements of methionine + cysteine; <sup>B</sup>All = diet formulated to meet the requirements of all amino acids: Met, Lys, Thr, Trp, Val, Arg, and Ile. Source: own research.

**Table 2.** Percentage composition of the experimental diets, on natural matter basis. Diets for the 1-21 d phase (Experiment 1).

Ingredients, %	Met+Cys	Met+Cys and Lys	Met+Cys and Thr	Met+Cys and Trp	Met+Cys and Val	Met+Cys and Arg	Met+Cys and Ile	All <sup>B</sup>
Corn	68.16	67.78	67.88	70.51	68.09	68.13	67.96	69.27
Soybean	27.90	27.94	27.93	25.50	27.91	27.90	27.92	25.64
Limestone	1.24	1.24	1.24	1.25	1.24	1.24	1.24	1.25
Phosphate <sup>A</sup>	1.16	1.17	1.17	1.18	1.16	1.16	1.16	1.18
Comum Salt	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Vitamin mix <sup>C</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral mix <sup>D</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Inert <sup>E</sup>	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Antioxidant <sup>F</sup>	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
DL-Met (99%)	0.27	0.27	0.27	0.29	0.27	0.27	0.27	0.29
L-Lys-HCL	---	0.34	---	---	---	---	---	0.39
L-Thr (98%)	---	---	0.25	---	---	---	---	0.28
L-Trp (99%)	---	---	---	0.01	---	---	---	0.01
L-Val (99%)	---	---	---	---	0.06	---	---	0.10
L-Arg (99%)	---	---	---	---	---	0.03	---	0.10
L-Ile (98%)	---	---	---	---	---	---	0.18	0.22
Total	100	100	100	100	100	100	100	100
<i>Chemical composition of the experimental diets</i>								
ME (kcal/kg)	2,900	2,900	2,900	2,900	2,900	2,900	2,900	2,900
Ca (%)	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
AvP (%)	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
CP (%)	18.9	19.2	19.1	18.0	18.9	18.9	19.0	18.9
	(17.3)	(17.4)	(16.7)	(16.8)	(17.3)	(17.3)	(17.6)	(17.6)
Met+Cyst (%)	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
	(0.80)	(0.77)	(0.86)	(0.85)	(0.83)	(0.79)	(0.83)	(0.85)
Lys (%)	0.85	1.19	0.85	0.80	0.85	0.85	0.85	1.19
	(0.95)	(1.13)	(0.90)	(0.90)	(0.93)	(0.90)	(0.90)	(1.15)
Thr (%)	0.62	0.62	0.87	0.59	0.62	0.62	0.62	0.87
	(0.68)	(0.66)	(0.90)	(0.66)	(0.66)	(0.66)	(0.66)	(0.89)
Trp (%)	0.18	0.18	0.18	0.20	0.18	0.18	0.18	0.20
Val (%)	0.78	0.78	0.78	0.74	0.84	0.78	0.78	0.84
	(0.85)	(0.82)	(0.80)	(0.81)	(0.90)	(0.83)	(0.82)	(0.85)
Arg (%)	1.14	1.14	1.14	1.07	1.14	1.16	1.14	1.16
	(1.16)	(1.12)	(1.10)	(1.12)	(1.15)	(1.16)	(1.12)	(1.13)
Ile (%)	0.71	0.71	0.71	0.67	0.71	0.71	0.89	0.89
	(0.74)	(0.71)	(0.70)	(0.70)	(0.74)	(0.72)	(0.89)	(0.88)

<sup>A</sup>Dicalcium phosphate: 18.5% P and 24.5% Ca; <sup>B</sup>Diet was formulated to meet the demands of all amino acids in question; <sup>C</sup>Supplied per kilogram of diet: vitamin A, 8,818 IU; vitamin D3, 2,646 ICU; vitamin E, 22 IU; vitamin B12, 26 µg; riboflavin, 8.8 mg; niacin, 88 mg; d-pantothenic acid, 22 mg; vitamin K, 2.6 mg; folic acid, 2.2 mg; vitamin B6, 4.3 mg; thiamine, 3.7 mg; d-biotin, 220 µg; <sup>D</sup>Supplied per kilogram of diet: iron (ferrous sulphate), 40 mg; manganese (manganese sulphate and manganous oxide), 120 mg; zinc (zinc oxide), 210 mg; cobalt (cobalt carbonate), 2.2 mg; iodine (calcium iodate), 132 mg; <sup>E</sup>Washed sand; <sup>F</sup>Ethoxyquin feed; Values in parentheses are the analysed composition. Source: own research.

**Table 3.** Percentage composition of the experimental diets, on natural matter basis.  
Diets for the 22-42 d phase (Experiment 2).

Ingredients, %	Met+Cys	Met+Cy: and Lys	Met+Cys and Thr	Met+Cys and Trp	Met+Cys and Val	Met+Cys and Arg	Met+Cys and Ile	All <sup>B</sup>
Corn	78.39	77.91	78.02	80.74	78.26	78.16	78.18	79.01
Soybean	17.87	17.93	17.91	15.48	17.89	17.90	17.90	15.67
Limestone	1.07	1.07	1.07	1.08	1.07	1.07	1.07	1.07
Phosphate <sup>A</sup>	1.11	1.11	1.11	1.12	1.11	1.11	1.11	1.13
Comum Salt	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Vitamin mix <sup>C</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral mix <sup>D</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Inert <sup>E</sup>	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Antioxidant <sup>F</sup>	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
DL-Met	0.29	0.29	0.29	0.31	0.29	0.29	0.29	0.31
L-Lys-HCL	---	0.43	---	---	---	---	---	0.49
L-Thr (98%)	---	---	0.33	---	---	---	---	0.36
L-Trp (99%)	---	---	---	0.01	---	---	---	0.01
L-Val (99%)	---	---	---	---	0.12	---	---	0.16
L-Arg	---	---	---	---	---	0.21	---	0.29
L-Ile (98%)	---	---	---	---	---	---	0.19	0.23
Total	100	100	100	100	100	100	100	100
<i>Chemical composition of the experimental diets</i>								
ME	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050
Ca (%)	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
AvP (%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
CP (%)	15.33	15.78	15.57	14.47	15.41	15.32	15.45	15.51
	(13.8)	(14.1)	(13.9)	(13.1)	(13.7)	(14)	(16.7)	(14.7)
Met+Cyst	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
(%)	0.77)	(0.75)	(0.70)	(0.71)	(0.72)	(0.74)	(0.78)	(0.79)
Lys (%)	0.62	1.05	0.62	0.56	0.62	0.62	0.62	1.05
	(0.70)	(0.97)	(0.68)	(0.62)	(0.66)	(0.66)	(0.74)	(1.15)
Thr (%)	0.49	0.49	0.82	0.46	0.49	0.49	0.49	0.82
	(0.52)	(0.53)	(0.80)	(0.50)	(0.52)	(0.51)	(0.55)	(0.90)
Trp (%)	0.14	0.14	0.14	0.15	0.14	0.14	0.14	0.15
Val (%)	0.62	0.62	0.62	0.58	0.74	0.62	0.62	0.74
	(0.67)	(0.66)	(0.66)	(0.63)	(0.75)	(0.65)	(0.70)	(0.80)
Arg (%)	0.85	0.85	0.85	0.79	0.85	1.05	0.85	1.05
	(0.87)	(0.85)	(0.85)	(0.80)	(0.84)	(1.06)	(0.91)	(1.14)
Ile (%)	0.55	0.55	0.55	0.51	0.55	0.55	0.74	0.74
	(0.56)	(0.55)	(0.55)	(0.52)	(0.55)	(0.54)	(0.78)	(0.79)

<sup>A</sup>Dicalcium phosphate: 18.5% P and 24.5% Ca; <sup>B</sup>Diet was formulated to meet the demands of all the amino acids in question; <sup>C</sup>Supplied per kilogram of diet: vitamin A, 8,818 IU; vitamin D3, 2,646 ICU; vitamin E, 22 IU; vitamin B12, 26 µg; riboflavin, 8.8 mg; niacin, 88 mg; d-pantothenic acid, 22 mg; vitamin K, 2.6 mg; folic acid, 2.2 mg; vitamin B6, 4.3 mg; thiamine, 3.7 mg; d-biotin, 220 µg; <sup>D</sup>Supplied per kilogram of diet: iron (ferrous sulphate), 40 mg; manganese (manganese sulphate and manganese oxide), 120 mg; zinc (zinc oxide), 210 mg; cobalt (cobalt carbonate), 2.2 mg; iodine (calcium iodate), 132 mg; <sup>E</sup>Washed sand; <sup>F</sup>Ethoxyquin feed; Values in parentheses are the analysed composition.

Source: own research.



**Table 4.** Percentage composition of the experimental diets, on natural matter basis. Diets for the egg laying phase (Experiment 3).

Ingredients, %	Met+Cys	Met+Cys and Lys	Met+Cys and Thr	Met+Cys and Trp	Met+Cys and Val	Met+Cys and Arg	Met+Cys and Ile	All <sup>B</sup>
Corn	64.22	64.23	64.22	64.22	64.22	64.22	64.22	64.22
Soybean	25.50	25.50	25.50	25.50	25.50	25.50	25.50	25.50
Limestone	7.09	7.09	7.09	7.09	7.09	7.09	7.09	7.09
Phosphate <sup>A</sup>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Comum Salt	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53
Corn starch	1.21	0.79	1.14	1.20	1.00	1.00	0.85	---
Vitamin mix <sup>C</sup>	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Mineral mix <sup>D</sup>	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Inert <sup>E</sup>	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antioxidant <sup>F</sup>	0.06	0.03	0.03	0.07	0.11	0.03	0.19	0.10
K <sub>2</sub> CO <sub>3</sub>	---	0.12	---	---	---	---	---	0.12
DL-Met	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
L-Lys-HCL	---	0.32	---	---	---	---	---	0.32
L-Thr (98%)	---	---	0.10	---	---	---	---	0.10
L-Trp (99%)	---	---	---	0.00	---	---	---	0.00
L-Val (99%)	---	---	---	---	0.16	---	---	0.16
L-Arg (99%)	---	---	---	---	---	0.24	---	0.24
L-Ile (98%)	---	---	---	---	---	---	0.23	0.23
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>

*Chemical composition of the experimental diets*

ME (kcal/kg)	2,800	2,800	2,800	2,800	2,800	2,800	2,800	2,800
Ca (%)	3.05	3.05	3.05	3.05	3.05	3.05	3.05	3.05
AvP (%)	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
CP (%)	16.73	17.01	16.81	16.73	16.86	17.17	16.89	17.80
	(16.7)	(16.7)	(17)	(16.8)	(17.6)	(18.1)	(17.5)	(18.8)
Met+Cyst	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
(%)	(0.74)	(0.74)	(0.73)	(0.74)	(0.78)	(0.74)	(0.78)	(0.75)
Lys (%)	0.78	1.03	0.78	0.78	0.78	0.78	0.78	1.03
	(0.86)	(1.11)	(0.83)	(0.84)	(0.82)	(0.85)	(0.82)	(1.10)
Thr (%)	0.57	0.57	0.67	0.57	0.57	0.57	0.57	0.67
	(0.64)	(0.64)	(0.73)	(0.63)	(0.65)	(0.67)	(0.66)	(0.74)
Trp (%)	0.16	0.16	0.16	0.18	0.16	0.16	0.16	0.18
Val (%)	0.71	0.71	0.71	0.71	0.87	0.71	0.71	0.87
	(0.81)	(0.81)	(0.82)	(0.80)	(0.97)	(0.81)	(0.81)	(0.97)
Arg (%)	1.03	1.03	1.03	1.03	1.03	1.26	1.03	1.26
	(1.08)	(1.10)	(1.11)	(1.05)	(1.10)	(1.31)	(1.10)	(1.30)
Ile (%)	0.64	0.64	0.64	0.64	0.64	0.64	0.87	0.87
	(0.71)	(0.71)	(0.72)	(0.70)	(0.71)	(0.75)	(0.93)	(0.95)

<sup>A</sup>Dicalcium phosphate: 18.5% f P and 24.5% Ca; <sup>B</sup>Diet was formulated to meet the demands of all the amino acids in question; <sup>C</sup>Supplied per kilogram of diet: vitamin A, 8,818 IU; vitamin D3, 2,646 ICU; vitamin E, 22 IU; vitamin B12, 26 µg; riboflavin, 8.8 mg; niacin, 88 mg; d-pantothenic acid, 22 mg; vitamin K, 2.6 mg; folic acid, 2.2 mg; vitamin B6, 4.3 mg; thiamine, 3.7 mg; d-biotin, 220 µg; <sup>D</sup>Supplied per kilogram of diet: iron (ferrous sulphate), 40 mg; manganese (manganese sulphate and manganous oxide), 120 mg; zinc (zinc oxide), 210 mg; cobalt (cobalt carbonate), 2.2 mg; iodine (calcium iodate), 132 mg; <sup>E</sup>Washed sand; <sup>F</sup>Ethoxyquin feed; Values in parentheses are the analysed composition. Source: own research.

### ***Experiments 1 and 2***

A total of 480 one-day-old female Japanese quail were used in experiment 1 (1-21-day-old quail). In experiment 2, 480 different birds were used in the 21<sup>st</sup> to 42<sup>nd</sup> days of life (22-42 days old). The birds were housed in galvanized wire cages (70 × 50 × 30 cm) equipped with trough feeders and drinkers. Ten-day-old birds were vaccinated against Newcastle virus through the drinking water. Marek's vaccine was administered at the hatchery. The lighting programme was 17L:7D until the 12<sup>th</sup> day; then, the birds were subjected to a 17L photoperiod. Feed and water were provided *ad libitum* throughout the experimental period.

The variables evaluated were feed intake (FI, g/bird), body weight gain (BWG, g/bird) and feed conversion ratio (FCR, kg/kg). The feed leftovers were weighed and used to calculate the FI. BWG was calculated as the mean difference in body weight measured at the beginning and end of the experimental period per replicate.

### ***Experiment 3***

A total of 480 quail (85 to 210 days old) with an initial egg production of 96.3% ± 0.68% were used in experiment 3, which was divided into five periods of 21 days each for data collection. The birds were housed in galvanized wire cages (33 × 33 × 14 cm) equipped with trough feeders and drinkers. The photoperiod used was 17L:7D. Feed and water were provided *ad libitum* throughout the experimental period.

The variables evaluated at the end of each 21-day period were FI (g/bird), egg-production (EP, %), mean egg weight (EW, g), egg mass (EM, g), feed conversion per egg mass (CEM, kg of feed/kg of eggs), feed conversion per dozen eggs (CDZ, kg of feed/dozen eggs), relative weight of the egg yolk (WYolk, g), albumen (WAlb, g), shell weight (WShell, g), shell thickness (SThick, mm) and specific gravity (SG, g/cm).

Egg production was recorded daily (8:00 A.M.), and the mean egg production per quail per day was calculated per experimental unit. The mean egg weight was determined by collecting all intact eggs on the 19<sup>th</sup>, 20<sup>th</sup> and 21<sup>st</sup> days of each 21-day period and was calculated by dividing the total weight of the collected eggs by the number of collected eggs for each experimental unit. The egg mass was calculated by multiplying the mean egg weight by the total number of eggs produced, and the result was divided by the total number of quail per period. The egg mass was expressed as grams per quail per day. Feed conversion was

calculated by dividing the feed intake by the egg production per dozen eggs or the egg production in kilograms of eggs per period.

To determine the weight of the egg components (yolk, albumen and shell), three eggs were selected based on the mean weight. The eggs were individually weighed in a 0.01<sup>g</sup> precision scale, labelled and then broken. The egg yolk was weighed, and the eggshells were dried at 105°C for 4 hours and then weighed. The albumen weight was determined by subtracting the yolk and shell weights from the original egg weight.

The eggshell thickness was measured around the equator of the egg with the aid of a digital micrometre with 0.1 mm accuracy. The specific gravity was determined in 15 saline solutions (varying density by 0.0025 units starting at 1.0625<sup>-1</sup>.100) according to the method described by Hamilton (1982); two independent eggs were selected per experimental unit per day.

### ***Statistical analysis***

The normality of the data was verified via the skewness and kurtosis coefficients. All data were analysed by analysis of variance (ANOVA) using the general linear model procedures in SAS (SAS Institute Inc., Carey, NC, USA). Means were compared using the Student-Newman-Keuls (SNK) test. The minimum confidence level was  $P < 0.05$ .

## **3. Results and discussion**

### ***Experiments 1 and 2***

There were no significant differences between the treatments in FI ( $P > 0.05$ ) for the performance results for quail aged 1 to 21 days (Experiment 1, Table 5) and 22 to 42 days (Experiment 2, Table 5). The BWG and FCR of animals of both age groups were significantly affected by the studied diets ( $P < 0.05$ ). The highest BWG and the best FCR were observed in birds fed diets that met the Met+Cys and Lys requirements.

In experiments 1 and 2 (Table 5), meeting the Lys requirement led to higher BWG ( $P = 0.0001$ ) and better FCR ( $P = 0.0001$ ). Birds aged 1-21 days fed diets that met the Met+Cys and Lys requirements showed higher BWG (4 g/bird) and better FCR (5.11%) than birds fed diets that only met the Met+Cys requirements (BWG = 83 vs 79 g) (FCR = 2.54 vs 2.68 g/g).

The performance of 22- to 42-day-old quail fed diets with adequate levels of Met+Cys and Lys was followed by the performance of birds fed diets that met the requirements for all AAs (Met+Cys, Lys, Thr, Trp, Val, Arg and Ile), with a BWG of 8 g/bird (72 vs 64 g) and a 13.62% improvement in the FCR (4.5 vs 5.21%). An inverse result was observed for bird performance according to the AA requirements. The diet that provided the second best performance (Met+Cys) in younger birds (1-21 days old) was the third best diet for the older birds (22-42 days old); fulfilling the requirements of all studied AAs (Met+Cys, Lys, Thr, Trp, Val, Arg and Ile) became more important for the older quail (22-42 days old). Diets that met the requirements of Met+Cys and the other AAs (Thr, Trp, Val, Arg and Ile) individually limited the bird performance and led to the poorest BWG and FCR results. The diet that met the Met+Cys and Trp requirements led to the greatest limitation in the growth and performance of birds in both age groups (Experiments 1 and 2).

**Table 5.** Feed intake (FI), body weight gain (BWG) and Feed conversion ratio (FCR) of 1- to 21-day-old (Experiment 1) and 22- to 42-day-old (Experiment 2) Japanese quail fed diets with adequate levels of different amino acids.

AA Requirement met	1-21 d (Experiment 1)			22-42 d (Experiment 2)		
	FI (g/bird)	BWG (g/bird)	FCR (g/g)	FI (g/ bird)	BWG (g/ bird)	FCR (g/g)
Met+Cys <sup>A</sup>	213	79 <sup>b</sup>	2.68 <sup>b</sup>	328	60 <sup>c</sup>	5.47 <sup>bc</sup>
Met+Cys and Lys	211	83 <sup>a</sup>	2.54 <sup>a</sup>	324	72 <sup>a</sup>	4.50 <sup>a</sup>
Met+Cys and Thr	217	71 <sup>d</sup>	3.07 <sup>c</sup>	329	50 <sup>d</sup>	6.50 <sup>d</sup>
Met+Cys and Trp	215	64 <sup>e</sup>	3.34 <sup>d</sup>	336	51 <sup>d</sup>	6.48 <sup>d</sup>
Met+Cys and Val	220	72 <sup>cd</sup>	3.06 <sup>c</sup>	329	56 <sup>c</sup>	5.83 <sup>c</sup>
Met+Cys and Arg	218	74 <sup>cd</sup>	2.95 <sup>c</sup>	331	57 <sup>c</sup>	5.77 <sup>c</sup>
Met+Cys and Ile	222	73 <sup>cd</sup>	3.03 <sup>c</sup>	327	56 <sup>c</sup>	5.77 <sup>c</sup>
All <sup>B</sup>	214	75 <sup>c</sup>	2.84 <sup>c</sup>	333	64 <sup>b</sup>	5.21 <sup>b</sup>
SEM	1.216	0.454	0.828	1.645	0.343	0.038
<i>P value</i>	0.4350	0.0001	0.0001	0.4776	0.0001	0.0001

<sup>A</sup>Control diet = diet with an adequate methionine level formulated to meet the requirements of methionine + cysteine; <sup>B</sup>All = diet formulated to meet the requirements of Met+Cys, Lys, Thr, Trp, Val, Arg, and Ile; <sup>a, b, c, d</sup> Different letters indicate significant differences between means according to the SNK test ( $P < 0.05$ ).

Source: own research.

Our study clearly showed that Lys was the limiting factor for bird performance in both experiments because all diets had adequate Met+Cys levels. Bird performance was improved by diets that had adequate levels of Lys in addition to Met+Cys, making Lys the second-limiting AA after Met+Cys. The Met+Cys and Lys levels tested in our study were in accordance with those used by Alagawany, et al. (2014). These authors performed an

experiment with a  $2 \times 2 \times 2$  factorial scheme with two CP levels (20 and 22%), two Lys levels (1.05 and 1.15%) and two total sulphur amino acid (TSAA) levels (0.8 and 0.9%) to investigate their effects on the performance and carcass characteristics of 7 to 42-day-old Japanese quail. The authors recommended diets containing 20% CP, 1.05% Lys and 0.8% TSAA.

We observed that quail fed diets that met the requirements for Met+Cys, Met+Cys and Thr, Met+Cys and Trp, Met+Cys and Val, Met+Cys and Arg, and Met+Cys and Ile fulfilled only 57.8% of the Lys requirement (0.853% *vs* 1.19%) recommended by the Table for Japanese and European quail (Silva & Costa, 2009).

Regarding the order of limiting AAs for quail, Mandal, et al. (2005), Parvin, et al. (2009), and Vieira, et al. (2017) cited Met as the first limiting AA. Information about the limiting order is scarce. In our study, Lys was the next AA to limit the performance of Japanese quail aged up to 42 days. Lys is an essential AA for growth and muscle development. According to Costa, et al. (2001), Lys is specifically associated with increased protein deposition and maintenance and, consequently, increased body weight and is used to a lesser extent in other metabolic processes. In cases of Lys deficiency, protein synthesis and, consequently, protein deposition are reduced (Gonzales, et al., 2002).

Furthermore, Lys participates in the synthesis of L-carnitine, which is a substance that is essential for energy use in the body (Nelson & Cox, 2014). Silva, et al. (2000) concluded that when a diet for replacement pullets had inadequate levels of Lys, the consequences on performance during the egg-laying phase were negative and irreversible. Lys deficiency in early life reduced breast muscle development (Tesseraud, et al. 1996) and increased protein degradation in the pectoralis major muscle during the growth period (Tesseraud, et al. 2009). Hasanvand, et al. (2017) observed that levels below 1.24% Lys, limited the development of quails.

### ***Experiment 3***

#### ***Performance***

The FI, EL, EM, CEM and CDZ of birds in the egg-laying phase were affected by the studied diets ( $P < 0.05$ ) (Table 6). No significant difference was detected for the EW ( $P > 0.05$ ).

In contrast to the growth phase (Experiments 1 and 2), the results observed in the egg-laying phase were not clear regarding the second-limiting AA. The birds fed diets that met the

Met+Cys and all AA requirements (especially Met+Cys and Lys, Met+Cys and Thr, Met+Cys and Trp, Met+Cys and Val, and Met+Cys and Arg) had the same EL rates. However, the diet with adequate Met+Cys and Ile levels led to the worst performance ( $P = 0.0002$ ).

The adequate supply of Met+Cys levels seems to be the most critical point in the formulation of practical diets for Japanese quail, which are primarily composed of maize and soybean meals. Only meeting the Met+Cys requirement was sufficient to maintain the same EL observed in the other treatments. Several studies evaluated the Met+Cys levels for Japanese quail (Pinto, et al., 2003; Garcia, et al., 2005; Reis, et al., 2009 and Scottá, et al., 2011) and demonstrated their positive influence on the EL. Costa, et al. (2009) observed that increased Met+Cys levels positively affected the EL, EW and EM and, consequently, improved the feed conversion per egg mass and per dozen eggs. These authors recommended a Met+Cys level of 0.667%.

**Table 6.** Egg production (EP), egg weight (EW), egg mass (EM), fee intake (FI), feed conversion by egg mass (CEM) and feed conversion by dozen eggs (CDZ) of Japanese quail in the egg-laying phase (Experiment 3) fed diets with adequate levels of different amino acids.

AA requirement met	EP (%)	EW (g)	EM (g)	FI (g/bird/d)	CEM (kg feed/kg egg)	CDZ (kg feed/dozen of egg)
Met+Cys <sup>A</sup>	87 <sup>a</sup>	11	9.8 <sup>ab</sup>	24 <sup>a</sup>	2.49 <sup>a</sup>	0.337 <sup>a</sup>
Met+Cys and Lys	90 <sup>a</sup>	11	10 <sup>ab</sup>	25 <sup>a</sup>	2.50 <sup>a</sup>	0.334 <sup>a</sup>
Met+Cys and Thr	87 <sup>a</sup>	11	9.7 <sup>b</sup>	25 <sup>a</sup>	2.60 <sup>a</sup>	0.346 <sup>a</sup>
Met+Cys and Trp	90 <sup>a</sup>	10	9.9 <sup>ab</sup>	25 <sup>a</sup>	2.52 <sup>a</sup>	0.331 <sup>a</sup>
Met+Cys and Val	86 <sup>a</sup>	11	9.6 <sup>b</sup>	24 <sup>ab</sup>	2.53 <sup>a</sup>	0.336 <sup>a</sup>
Met+Cys and Arg	87 <sup>a</sup>	11	9.9 <sup>ab</sup>	25 <sup>a</sup>	2.57 <sup>a</sup>	0.351 <sup>a</sup>
Met+Cys and Ile	83 <sup>b</sup>	11	9.4 <sup>b</sup>	24 <sup>ab</sup>	2.59 <sup>a</sup>	0.351 <sup>a</sup>
All <sup>B</sup>	91 <sup>a</sup>	11	10.4 <sup>a</sup>	22 <sup>b</sup>	2.20 <sup>b</sup>	0.302 <sup>b</sup>
SEM	0.41	0.04	0.05	0.14	0.01	0.002
<i>P value</i>	0.0002	0.1483	0.0016	0.002	0.0001	0.0004

<sup>A</sup>Control diet = diet with an adequate methionine level formulated to meet the requirements of methionine + cysteine; <sup>B</sup>All = diet formulated to meet the requirements of Met+Cys, Lys, Thr, Trp, Val, Arg, and Ile; <sup>a, b</sup>. Different letters indicate significant differences between means according to the SNK test ( $P < 0.05$ ); Source: own research.

As shown in the table 6, although the Met+Cys levels were important to maintain the EL ( $P = 0.0002$ ), the EM ( $P = 0.0016$ ) and the feed conversion per egg mass ( $P = 0.0001$ ) and per dozen eggs ( $P = 0.0004$ ), diets with adequate levels of all AAs studied (Met+Cys, Lys, Thr, Trp, Val, Arg and Ile) provided the best performance when the feed conversion per egg mass and number of eggs produced were considered. When the AA requirements are met,

protein synthesis for maintenance is maximal; therefore, more protein accumulates in the eggs. Furthermore, a greater amount of nitrogen from the CP is available for the synthesis of non-essential AAs with supplementation of essential AAs. When the requirement for the limiting nutrient (e.g., essential AAs) is met, the animals respond and improve their performance until their genetic potential is fully expressed (Brugalli, 2003).

In the our experiment, birds fed diets with adequate levels of all AAs (Met+Cys, Lys, Thr, Trp, Val, Arg and Ile) presents lowest FI (22.96 g/bird/d) ( $P = 0.002$ ). According to Kumta & Harper (1961), adequate levels of AAs in the plasma serve as a signal for appetite control.

### **Egg quality**

The diets affected the WYolk ( $P = 0.0001$ ) and Walb ( $P = 0.0005$ ) but did not affect the WShell ( $P = 0.5354$ ), Sthick ( $P = 0.0703$ ) and SG ( $P = 0.514$ ) (Table 7).

**Table 7.** Weight of egg yolk (WYolk), weight of egg albumen (WAlb), weight of eggshell (WShell), eggshell thickness (Sthick), and specific gravity (SG) of Japanese quail in the egg-laying phase (Experiment 3) fed diets with adequate levels of different amino acids.

AA requirement met	WYolk (g)	WAlb (g)	WShell (g)	Sthick (mm)	SG (g/cm <sup>3</sup> )
Met+Cys <sup>A</sup>	3.50 <sup>a</sup>	6 <sup>b</sup>	0.94	183	1.071
Met+Cys and Lys	3.63 <sup>a</sup>	6 <sup>b</sup>	0.95	185	1.075
Met+Cys and Thr	3.39 <sup>a</sup>	6 <sup>b</sup>	0.94	185	1.040
Met+Cys and Trp	3.45 <sup>a</sup>	6 <sup>b</sup>	0.95	188	1.071
Met+Cys and Val	3.39 <sup>a</sup>	6 <sup>b</sup>	0.95	182	1.066
Met+Cys and Arg	3.48 <sup>a</sup>	6 <sup>b</sup>	0.95	183	1.066
Met+Cys and Ile	3.54 <sup>a</sup>	6 <sup>b</sup>	0.96	181	1.071
All <sup>B</sup>	3.08 <sup>b</sup>	7 <sup>a</sup>	0.97	182	1.061
SEM	0.020	0.043	0.004	0.515	0.003
<i>P value</i>	0.0001	0.0005	0.5354	0.0703	0.514

<sup>A</sup>Control diet = diet with an adequate methionine level formulated to meet the requirements of methionine + cysteine; <sup>B</sup>All = diet formulated to meet the requirements of Met+Cys, Lys, Thr, Trp, Val, Arg, and Ile; <sup>a, b</sup>. Different letters indicate significant differences between means according to the SNK test ( $P < 0.05$ ); Source: own research.

The eggs of birds fed diets with adequate levels of all of the essential AAs studied (Met+Cys, Lys, Thr, Trp, Val, Arg and Ile) had a lower WYolk (approximately 27% of the egg weight) than the eggs of birds fed the other diets (30.44% of the total egg weight). Conversely, a higher WAlb was observed in the eggs of birds fed the minimum levels of all AAs. The availability of essential AAs possibly favored greater albumen synthesis and,



consequently, higher deposition in the eggs. Contrary to our findings, Ji, et al. (2014) reported that diets with reduced CP and no supplementation of crystalline AAs favored the production of eggs with reduced albumen percentages and higher egg yolk percentages. The authors suggest that the AA necessary for albumen synthesis may have been limited at low CP levels.

#### **4. Conclusion**

The second-limiting amino acid in corn-soybean meal based diets for Japanese quail in the growth phase (1-42 days of age) is lysine, but do not to egg-laying phase; however, diets with adequate levels of methionine+cysteine are sufficient to maintain egg production without causing losses.

We recommend further studies to define the second-limiting amino acid to egg-laying phase, and the sequence order limiting amino acid in corn-soybean meal and animal products based diets to Japanese quails.

#### **Conflict of interest**

The authors declare that there is no conflict of interest.

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