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Requirement of digestible methionine + cystine in Japanese quails during the laying phase

Exigência de metionina + cistina digestível para codornas japonesas em fase de postura

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Abstract
An experiment was carried out with Japanese quails in the initial laying phase, from 43 to 168 days of age, to determine the nutritional requirement of digestible methionine + cystine for this period. 375 quails were used, being fifteen quails used per experimental unit. A total of 5 treatments (0.60, 0.75, 0.90, 1.05 and 1.20% digestible methionine + cystine) were used in a completely randomized design with 5 replicates each. The performance parameters evaluated were feed intake (g / bird), body weight (g), egg weight (g), laying rate (%), egg mass (g eggs.bird.day-^{1}), feed conversion by mass and dozen eggs (g.g-^{1} of eggs, g.dz-^{1} of eggs), viability (%) and the body chemical composition (%). The egg quality parameters were: % of component (yolk, albumen and shell relative to egg weight), specific gravity (g mL-^{1}), Haugh unit, yolk index, shell weight per surface area and thickness of the shell (mm). Quadratic effect was found on the performance parameters evaluated (P<0.05), except for age at first egg and viability with linear effect. Regarding egg quality, no significant effect was observed on the variables tested (P>0.05). The nutritional recommendation of digestible methionine + cystine for Japanese quails at laying phase is 0.90% from the maximum point obtained for the egg mass, egg weight and laying rate, corresponding to daily intake of 241.54 mg of digestible methionine + cystine / day, respectively.
Keywords: body composition; egg mass; egg quality; egg weight.

Resumo
Foi desenvolvido um experimento com codornas japonesas na fase inicial de postura, de 43 a 168 dias de idade, com o objetivo de determinar a exigência nutricional de metionina + cistina digestível para este período. Foram utilizadas 375 codornas, sendo 15 aves por unidade experimental. O delineamento experimental foi inteiramente casualizado (DIC) totalizando 5 tratamentos (0,60; 0,75; 0,90; 1,05 e 1,20 % de metionina + cistina digestível) com 5 repetições cada. As variáveis de desempenho avaliadas foram, consumo de ração (g/ave), peso corporal (g), peso do ovo (g), taxa de postura (%), produção de massa de ovos (g ovos.ave. dia^{-1}), conversão alimentar por massa e dúzia de ovos (g.g^{-1} de ovos, g.dz^{-1} de ovos), viabilidade (%) e a composição química corporal (%). As variáveis de qualidade dos ovos foram, percentagem do componente (gema, albúmen e casca em relação ao peso do ovo), gravidade específica (g mL.$^{-1}$), unidade Haugh, índice de gema e peso da casca por superfície de área. Foi encontrado efeito quadrático sobre as variáveis de desempenho avaliadas (P<0,05), exceto para idade ao primeiro ovo e viabilidade que apresentaram efeito linear. Em relação à qualidade dos ovos, não foi observado efeito significativo sobre as variáveis testadas (P>0,05). A recomendação nutricional de metionina + cistina digestível para codornas japonesas na fase de postura é de 0,90% a partir do ponto de máxima obtido para as variáveis massa de ovos, peso de ovos e taxa de postura, correspondendo ao consumo diário de 241,54 mg de metionina + cistina digestível / dia, respectivamente.
Palavras-chave: composição corporal; massa de ovos; peso do ovo; qualidade de ovos.

1.Introduction

Amino acids have important roles as the components of proteins, participate in several metabolic processes, and are essential for animal development and biological process maintenance[(1)]. The order of the limiting essential amino acids in poultry diets has been studied for several decades. According to D´Mello[(2)], the first limiting amino acids for most poultry diets are sulphur amino acids (methionine and cystine), followed by lysine and threonine.

Methionine is the first limiting amino acid in the diet because the main ingredients of feed, corn, and soybean meal, cannot supply the requirements for maintaining and growing these birds[(3)]. Therefore, adding small amounts of limiting amino acids significantly increases the quality of dietary protein. Cysteine is

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present in ingredients as well as other amino acids and is synthesised in the animal's body from methionine; thus, it is classified as a non-essential amino acid[(3)].

Both are involved in a series of biosynthetic pathways, generating intermediates of the cycle of citric acid, and acting in the formation of glutathione peroxidase, which is the most important body antioxidant system[(2)]. Cysteine also participates in the structure of many proteins such as insulin, immunoglobulins, and keratin by interconnecting polypeptide chains via disulphide bonds[(3)]. It has the function of stimulating the haematopoietic system, promoting the formation of white and red blood cells. Furthermore, when metabolised, cysteine provides sulphuric acid, which reacts with other substances to help detoxify the body and contributes to the process of healing and strengthening connective tissue[(4)].

These amino acids are normally supplemented in the birds' diet by adding the synthetic amino acid, DL-methionine, which is a metabolic precursor of cystine. Unlike most amino acids that are commercially produced by fermentation, crystalline methionine is produced by chemical synthesis. This has an important biological implication because fermentation produces only the L-isomer, whereas chemical synthesis produces a racemic mixture of D and L isomers[(5)].

However, there is still insufficient information on the nutritional requirements of quails; therefore, research regarding specific nutrition and feeding programmes for each stage of development of quails is critically required. Reports from such studies will allow the optimisation of the productive potential of this species, which is gaining importance because of its prolific egg production capacity, high growth rate, and resistance to diseases.

Therefore, the objective of this study was to determine the nutritional requirement of digestible methionine + cystine for Japanese quail (*Coturnix coturnix japonica*) in the starter stages of laying, from 43 to 168 days of age, for maximum performance and egg quality.

2. Materials and methods

The experimental procedures were approved by the Ethics and Biosafety Committee of the State University of Maringá (Protocol Number 9195040417).

2.1. Animals, diets, and experimental design

The experiments were performed in Maringá, Paraná, Brazil (Latitude: 23 o 25' 38" South, Longitude: 51 o 56' 15" West) from July to December 2015. The experimental period was based on the age of quails (from 43 to 168 days). However, for statistical analysis, performance and egg quality parameters were evaluated from the 64th day of life (divided into five cycles of 21 days each), at which point egg production becomes homogeneous.

One-day-old birds, purchased from a commercial source (Vicami – Assis-SP, Brazil), were raised until 42 days old in a conventional shed. During this phase, they received a feed formulated to meet the requirements proposed by Rostagno et al.[(6)] for Japanese quails in the growth stages. On the 43rd day, these birds were transferred to a conventional laying shed with a clay tile roof, floor, and masonry sidewalls (height 0.50 m), with wire mesh up to the roof, side curtains, nipple-type drinking fountains, and trough-type troughs in galvanised wire cages.

The experimental design was completely randomised (DIC) with five treatments (0.60%, 0.75%, 0.90%, 1.05%, and 1.20% methionine + cystine) with five replicates for each treatment. Fifteen female quails were used per experimental unit (cage), totalling 375 birds.

In the formulation of the experimental feed, the chemical composition values of the ingredients proposed by Rostagno et al.[(6)] were considered except for corn and soybean meal values, which were previously determined in a specialised laboratory (Evonik Degussa Brasil Ltda.) (Table 1).

Table 1. Centesimal composition of experimental diets for Japanese quails in the laying phase

Ingredients (%)	Digestible methionine + cystine (%)			
	0.60	**0.75**	**0.90**	**1.05**
Corn grain	58.00	58.00	58.00	58.00
Soybean meal 45%	30.28	30.28	30.28	30.28
Glutamic acid	0.64	0.48	0.32	0.16
Soybean oil	1.35	1.32	1.29	1.26
Inert[1]	0.51	0.55	0.59	0.63
Monocalcium phosphate	1.07	1.07	1.07	1.07
Limestone	6.95	6.95	6.95	6.95
Suplemento vit/min[2]	0.40	0.40	0.40	0.40
Common salt	0.32	0.32	0.32	0.32
L-lysine	0.27	0.27	0.27	0.27
DL-methionine	0.09	0.24	0.39	0.54
L-threonine	0.07	0.07	0.07	0.07
L-tryptophan	0.04	0.04	0.04	0.04
Antioxidant[3]	0.01	0.01	0.01	0.01
Total (kg)	100.00	100.00	100.00	100.00

[1]Inert: sand. [2]Mineral/vitamin supplement (guarantee levels per kg product): Vit. A – 1.000.000 UI; Vit. D3 – 300,000 UI; Vit E – 2,000 UI; Vit. B1 – 250 mg; Vit. B2 – 600 mg; Vit. B6 – 500 mg; Vit. B12 – 2,000 mcg; Vit. K3 – 300 mg; Ca pantothenate – 1,200 mg; Niacin – 2,4000 mg; Folic acid – 100 mg; Biotin – 20 mg; Choline – 30 g C; Zn - 5 g; Fe – 5 g; Mn – 6 g; Cu – 1,200 mg; I – 100 mg; Co – 20 mg; Se – 25.2 mg. [3]BHT (Butyl Hydroxy Toluene)

The feeds were formulated to meet the requirements proposed by Rostagno et al.[(6)] for Japanese quails in the laying phase, except for the methionine +

cystine content (Table 2).

Table 2. Nutritional composition* of experimental diets for Japanese quails in the laying phase.

Nutrients	Digestible methionine + cystine (%)				
	0.60	0.75	0.90	1.05	1.20
ME[1] (kcal kg^{-1})	2,800	2,800	2,800	2,800	2,800
Crude protein (%)	18.8	18.8	18.8	18.8	18.8
Calcium (%)	2.92	2.92	2.92	2.92	2.92
Available phosphorus (%)	0.30	0.30	0.30	0.30	0.30
Digestible lysine (%)	1.09	1.09	1.09	1.09	1.09
Digestible threonine (%)	0.65	0.65	0.65	0.65	0.65
Digestible tryptophan (%)	0.23	0.23	0.23	0.23	0.23
Sodium (%)	0.14	0.14	0.14	0.14	0.14
Clorine (%)	0.29	0.29	0.29	0.29	0.29
Potassium (%)	0.72	0.72	0.72	0.72	0.72
EB[2] (mEq kg^{-1})	166.85	166.85	166.85	166.85	166.85

*Calculated values; [1]Metabolizable energy; [2]Electrolyte balance of diets being calculated according to Mongin (1981): EB = (mg/kg of Na^+ of the feed / 22,990) + (mg/kg of K^+ of the feed / 39,102) - (mg/kg of Cl^- of the feed / 35,453).

The feeds were also formulated with corn and soybean meal to become isocalphic, isophosphoric, isoenergetic, and isoaminoacidic, except for digestible methionine + cystine. The different digestible methionine + cystine levels of the feed were adjusted by varying the amounts of DL-methionine (99%), glutamic acid, soybean oil, and inert (sand). The amounts of other ingredients did not vary among the five treatments. To calculate the electrolytic balance of the experimental feeds (170 mEq/kg) the molecular weight of each chemical element was considered, in accordance with Mongin[7].

The minimum and maximum temperatures (18.61 °C and 28.92 °C) as well as relative humidity (61.18% and 88.01%, respectively) were recorded in the morning using maximum and minimum dry bulb thermometers. These thermometers were located at bird height and at two points in the shed. The light programme used from the 43rd day started with 14 hours of light. Gradually, 30 minutes of light were added weekly until a light cycle of 17 hours of natural + artificial light was obtained. This was controlled with the aid of a timer; a luminous intensity of 21 lux was used.

2.2, Performance parameters

For the evaluation of zootechnical performance, the birds were weighed at the end of each production cycle. The experimental feed provided as well as leftovers were also weighed to determine the feed intake (g/bird), body weight (g), conversion feed per egg mass (gg^{-1} of eggs), feed conversion per dozen eggs (g.dz^{-1} of eggs), and viability (%). The eggs were collected daily at 8:00 am to calculate the posture rate (%) and egg mass production (g eggs.bird.day^{-1}), counting all the eggs produced.

- Feed conversion per egg mass (gg^{-1} of eggs):

$$\text{FCEM (g.g}^{-1}\text{ of eggs)} = \frac{\text{daily feed intake (g)}}{\text{egg mass(g eggs.day}^{-1}\text{)}}$$

- Feed conversion per dozen eggs (g.dz^{-1} of eggs):

$$\text{FCDE (g.dz}^{-1}\text{ of eggs)} = \frac{\text{daily feed intake (g)}}{\text{dozen eggs(dz eggs.day}^{-1}\text{)}}$$

- Viability (%):

$$\text{VI(\%)} = \frac{\text{number of birds at the end of the experimental period}}{\text{number of birds at the beginning of the experimental period}} .100$$

- Laying rate (%):

$$\text{LR(\%)} = \frac{\text{number of eggs}}{\text{number of birds}} .100$$

- Egg mass (g eggs.bird.day^{-1}):

$$\text{EgM(g eggs.bird.day}^{-1}\text{)} = \frac{\text{laying rate (\%)}}{100} \text{.egg weigh (g)}$$

2.3. Body chemical composition

For the determination of body chemical composition, two birds at 168 days of age were selected according to the mean weight (± 5%) using the methodology described by Sakomura and Rostagno[1]. The selected birds were subjected to fasting for 5 hours and were then sacrificed by intravenous desensitisation using thiopental barbiturate (100 mg/kg) followed by cervical dislocation.

After slaughtering, they were frozen and subsequently ground in an industrial meat grinder. The samples were then homogenised, weighed, and put into a forced ventilation kiln at 55 °C for pre-drying. After 72 hours, the samples were removed from the kiln and weighed again. After pre-drying, the samples were grinded in a knife-type mill and taken to the laboratory for the determination of dry matter (DM - procedure no. 925,09), mineral matter (MM - procedure no. 923,03), crude protein (CP - procedure no. 920,87), and fat (CF procedure - no. 920,85), according to the methodologies described by AOAC[8].

To determine the protein deposition rate and body fat (FDR) ($g.day^{-1}$), two quails were slaughtered at 43 days of age for comparative analysis of the body chemical composition of the birds at 168 days. The amount of protein/fat in the final carcass (g) was determined by subtracting the amount of protein/fat in the initial carcass (g), divided by the experimental period (days), as described by Fraga[9]. To obtain the energy retained in the carcass (ERC), the equation described by Sakomura[10] was used; the equation was based on the energy values of the protein (5.66 Kcal/g) and fat (9.37 Kcal/g).

2.4. Egg quality

On the last 3 days of each cycle, all the eggs were identified and individually weighed on a digital precision scale (0.01 g). Then, three eggs that weighed within the mean (± 10%) weight of the respective treatment group were selected for internal and external quality analyses. Specific gravity was analysed for all collected eggs by immersing them in different concentrations of saline solution and adjusting the density using a Baumé densimeter ($0.005\ g.mL^{-1}$) from 1.060 to 1.085 $g.mL^{-1}$, according to the methodology described by Hamilton[11].

For the other analyses, the three eggs previously selected per replicate were used. These eggs were cross sectioned equatorially using surgical scissors, and the internal contents were arranged on a glass surface to measure the height (mm) and diameter (mm) of the yolk and density of albumen, using a digital calliper (Digimess®) with an accuracy of 0.02 mm. After the measurement, the yolk and albumen were separated to weigh the yolk on a precision scale. The weight of the albumen was obtained by subtracting the weight of the egg from the weights of the yolk and the shell. With the collected data, it was possible to determine several evaluation indices of the internal and external quality of the eggs.

- Yolk Index:

$$YI = \frac{\text{yolk height (mm)}}{\text{yolk diameter (mm)}}$$

- Unity Haugh, according Haugh[12]:

$$UH = 100 \log(A - (1.7.\ EW^{0.397}) + 7{,}57)$$

Being that, A = albumen height and EW = egg weight.

- % of component (% yolk, albumen and shell in relation to egg weight):

$$\%\text{ of component} = \frac{\text{weight of component (g)}}{\text{weight of egg (g)}}.100$$

- Shell weight per unit surface area (SWSA), adapted from Rodrigues et al.[13]:

$$SWSA = \frac{SW}{3.9782\ .\ EW^{0.7956}}.100$$

Being that, SW = shell weight (g) and EW = egg weight (g)

2.5. Statistical analysis

Statistical analysis of the data was performed using the statistical environment R (R Core Team, 2013)[14]. For the effects test (P <0.05), the model described below was adopted and then the assumption of the normality of the residues was verified. A significant effect of the factors (P <0.05) was determined by polynomial regression analysis of digestible methionine + cystine levels, with the aim of estimating the best-fit model of the data, according to Montgomery[15]. Estimates of the best level of methionine + cystine for each significant variable were obtained using the quadratic model, as proposed by Sakomura and Rostagno[1].

3. Results

The variables mean weight of birds, daily feed intake, egg weight, egg mass, laying rate, feed conversion per egg mass, and feed conversion per dozen eggs showed a quadratic effect (P <0.05), making it possible to estimate 0.90%, 0.90%, 0.88%, and 0.89% methionine + cystine digestible in the diet, respectively (Table 3).

However, both the age of the first egg and the viability decreased linearly (P <0.05) within the range of the levels tested. Therefore, it was not possible to estimate the optimal level for these variables. The variables of chemical composition and body deposition evaluated during the laying phase were as follows: crude fat in the carcass, rate of fat deposition, and ERC. These showed a linearly increasing effect (P <0.05) in response to the increase in methionine + cystine level in the feed. The other related variables did not present a significant effect (Table 4).

Regarding the parameters of internal and external egg quality, no significant effects (P >0.05) were observed for methionine + cystine supplementation (Table 5).

Table 3. Performance of Japanese quails in laying phase (8 to 24 weeks) as a function of digestible methionine + cystine levels.

M+C (%)	0.60	0.75	0.90	1.05	1.20	SE
WM (g)	177.67	170.18	171.91	170.33	172.59	0.62
DFI (g.bird.day^{-1})	27.00	26.81	27.17	26.88	28.61	0.16
EW (g)	10.42	10.88	11.10	10.92	10.46	0.06
EgM (g.bird.day^{-1})	8.58	9.56	9.88	9.92	8.50	0.14
LR (%)	82.31	87.81	88.97	90.85	81.30	0.97
FCEM (g. g egg^{-1})	3.15	2.81	2.75	2.71	3.37	0.06
FCDE (g. dz eggs^{-1})	0.47	0.40	0.40	0.40	0.49	0.01
AFE (days)	54.20	55.00	56.00	56.00	56.00	0.22
VI (%)	98.33	97.33	100.00	91.55	95.40	0.77
Regression				P value	R^2	Estimated M+C (%)
WM = 218.1332 – 9.8998 M+C +51.8414 M+C^2				<0.001 (Q)	0.66	0.96
DFI = 33.0591 – 16.0272 M+C +10.127 M+C^2				<0.001 (Q)	0.57	0.79
EW = 5.2285 + 12.9278 M+C -7.1429 M+C^2				<0.001 (Q)	0.89	0.90
EgM = -3.1735 + 29.16457 M+C -16.127 M+C^2				<0.001 (Q)	0.74	0.90
LR = 14.253 +168.6322 M+C -93.3016 M+C^2				0.001 (Q)	0.55	0.90
FCEM = 7.608 – 11.204 M+C +6.3556 M+C^2				<0.001 (Q)	0.75	0.88
FCDE = 1.18629 – 1.7971 M+C +1.0095 M+C^2				<0.001 (Q)	0.81	0.89
AFE = 52.6800 + 3.0667 M+C				0.001 (L)	0.38	-

M+C: digestible methionine + cystine; WM: mean weight of bird; DFI: daily feed intake; EW: egg weight; EgM: egg mass; LR: laying rate; FCEM: feed conversion per egg mass; FCDE: feed conversion per dozen eggs; AFE: age of the first egg; VI: viability; SE: standard error; Q: quadratic effect; L: linear effect.

Table 4. Body chemical composition[1] and proteins/fat deposition in Japanese quails in laying phase (8 to 24 weeks) as a function of digestible methionine + cystine levels

M+C (%)	0.60	0.75	0.90	1.05	1.20	SE
CP (%)	55.32	59.47	57.62	54.65	56.43	0.625
CF (%)	17.76	18.79	20.17	21.39	22.47	0.452
MM (%)	10.11	10.67	10.71	10.66	10.17	0.114
PDR (g.d^{-1})	0.03	0.03	0.03	0.03	0.04	0.003
FDR (g.d^{-1})	0.01	0.01	0.02	0.03	0.04	0.003
ERC (kcal.g^{-1})	0.26	0.28	0.37	0.47	0.54	0.036
Regression				P value	R^2	Estimated M+C (%)
CF = 12.9136+8.0026 M+C				<0.001 (L)	0.59	-
FDR = - 0.0176 + 0.0453 M+C				<0.001 (L)	0.59	-
ERC = - 0.0664 + 0.5026 M+C				0.0418 (L)	0.36	-

[1] Data presented in dry matter values. M+C: digestible methionine + cystine; CP: crude protein; CF: crude fat; MM: mineral matter; PDR: protein deposition rate; FDR: fat deposition rate; ERC: energy retained in the carcass; SE: standard error; L: linear effect.

Table 5. Egg quality of Japanese quail as a function of digestible methionine + cystine levels.

M+C (%)	0.60	0.75	0.90	1.05	1.20	SE
Albumen (%)	61.80	62.06	62.52	61.87	62.19	0.07
Shell (%)	7.46	7.62	7.31	7.38	7.44	0.03
Yolk (%)	30.34	30.38	30.10	30.77	30.41	0.08
SG (g.mL^{-1})	1.08	1.07	1.07	1.08	1.08	0.01
YI	0.47	0.48	0.48	0.48	0.47	0.01
SWSA (g/cm^2)	3.74	3.87	3.73	3.75	3.73	0.01
UH	94.14	92.85	93.30	93.55	93.16	0.17

M+C: digestible methionine + cystine; SG: specific gravity; YI: yolk index; SWSA: shell weight per unit surface area; UH: Unid Haugh; SE: standard error; Q: quadratic effect; L: linear effect.

4. Discussion

According to NRC[16] recommendations, Japanese quails should consume 0.70% total methionine + cystine, 1.00% total lysine, 20% crude protein, and 2,900 Kcal ME/kg during the laying phase. However, research has suggested that methionine levels above the NRC[16] recommendations may result in better performance[4, 6, 17]. The genetic composition of the birds used in this experiment is comes from the Southeast region of Brazil. This region is responsible for most of the number of quails (63,1%) and egg production (66,1%) in the national territory[32]. These birds had a mean weight of 173 g in the laying phase with an egg mass production peak of 9.92 g.bird.day^{-1}. Thus, the estimated nutritional requirement for quails weighing 177 g (medium weight) was 0.90% digestible methionine + cystine, as recommended by Rostagno et al.[6].

The Brazilian Tables for Poultry and Swine, Rostagno et al.[6] recommends 0.888%, 0.900%, and 0.857% of digestible methionine + cystine for birds weighing 165 g, 177 g, and 189 g, respectively. However, according to genetic improvement data reported by the Federal University of Viçosa, the quails used for determining the estimates presented in the tables of Rostagno et al.[17] presented higher average weights (190 g, 200 g, and 210 g) with a consequent higher requirement of digestible methionine + cystine (0.942%, 0.908%, and 0.869%, respectively,). These results are presented in comparison to the results of the previous publication.

It is known that larger birds are not synonymous with more productive birds. As can be seen in the tables by Rostagno et al.[17], birds with an average weight of 190 g presented higher egg mass (11 g/day) than birds weighing 200 g (10 g/day) and 210 g (9 g/day). According to Costa et al.[18], from genetic improvement in laying hens, they are becoming smaller and more productive because they efficiently convert the feed into an extremely rich and functional food, the egg. This is an important and fundamental advance for quail farming in Brazil, which does not yet have well-defined genetic material. Therefore, the recommendations are quite controversial regarding nutritional levels and stages of development[19].

Another important factor that should be evaluated when comparing the results of several studies is the crude protein level of the feed. According to Rostagno et al.[17], crude protein should be added to the feed to prevent birds from using the nitrogen derived from essential amino acids for synthesising non-essential amino acids, which can impair animal performance. These experimental feeds were formulated with 18.8% crude protein and 1.09% digestible lysine, as recommended by Rostagno et al.[6]. However, in other study observed in the literature[20], values ranging from 16.0 to 21.5% crude protein and 0.9 to 1.4% of digestible lysine, generating different recommendations for the other essential amino acids due to the intimate relationship of these in protein metabolism. In addition, as the protein content is reduced, non-essential nitrogen can become a limiting factor in feed [17].

Regarding the influence of methionine on egg production, some authors state that methionine is the amino acid that initiates protein synthesis and one of its main roles is to act on egg production, influencing weight and posture rate [18, 19]. According to Harms[21], methionine is an important amino acid in the control of egg weight, since the hens consume energy to support the number of eggs. However, the weight of eggs depends on the amino acid levels of the diet.

Considering the importance of methionine on egg production and the fact that egg mass is obtained from the relationship between the egg laying rate and egg weight, the level of methionine + cystine required to obtain a peak (EgM, LR, and EW) was the same (0.90%). This confirms the importance of these variables in estimating the required amount of digestible methionine + cystine for better productive performance. Some research has shown that protein and amino acid levels in peak production feeds influence egg size[22, 23]. Thus, increasing methionine levels relative to lysine in rations with adequate levels of the other essential amino acids is a viable and commonly used tool that provides greater egg production.

Reis et al.[4] and Costa et al.[18] also observed a quadratic effect for egg mass, obtaining a production peak at the level of 0.840% and 0.666% of digestible methionine + cystine in the diet, respectively. However, for Garcia et al.[24], there was no significant effect of methionine levels on this variable. The mass of eggs related to feed consumption, yielded an estimate of 0.88% of digestible methionine + cystine for food conversion by egg mass. According to Togashi et al.[25], supplementation with methionine in diets to satisfy the requirement of digestible methionine + cystine, favours the feed conversion because of the amino acid balance in the diets. This promotes the improved use of the nutrients and consequently improves their conversion into egg production. From this same variable, Reis et al.[4] obtained the estimate of 0.82%, while in the studies of Pinto et al.[26], Garcia et al.[24] and Scottá et al.[20], no significant effect was observed.

It is also important to evaluate the productivity of poultry per dozen eggs, as this determines their marketability. From the feed conversion (g.dz of eggs^{-1}), the maximum point at the level of 0.89% of methionine + cystine in the feed was obtained, which is close to that found for egg mass. In the work of Costa et al.[18], when evaluating feed conversion per dozen eggs, the level of methionine + cystine in the diet was estimated to be 0.683%, but Garcia et al.[24] and Reis et al.[4] did not find a significant effect for this variable.

According to Neme[27], the study of body composition is important for the definition of nutritional requirements, mainly of protein and fat deposition, as it helps to institute adequate feeding programmes to promote improvements in the productivity of the birds. When evaluating the chemical composition of the carcasses at 168 days of age, it was observed that there was an accumulation of fat in the carcass correlating with the increase in the levels of methionine + cystine in the feed. This was observed because the variables: crude fat in the carcass, rate of fat deposition, and ERC, had presented an increasing linear effect. As the bird ages, protein and energy requirements are altered due to the composition and rate of deposition of these in the body. However, the protein and energy ratio of the diet should be balanced so that a greater fat deposition does not interfere negatively in the cycle of egg production.

Despite the increasing deposition of adipose tissue, it did not negatively influence egg production as the methionine + cystine requirement could be consistently estimated from the production parameters. According to Macari et al.[28], in poultry, adipose tissues may be distributed in individualised deposits, such as those found in the abdominal region or less organised in other organs such as muscle, liver, skin, kidneys, and connective tissue.

It is interesting to note that despite directly influencing the lipid deposition, methionine did not influence the protein composition of the carcass. Kessler and Snizek[29] showed that protein deposition is closely controlled by genetics, and therefore there is a limit to their daily deposition, regardless of their intake. However, the amount of deposited fat is related to the amount of nutrients available for synthesis, regardless of the source.

Regarding egg quality, Shafer et al.[30] stated that the internal components of the egg are almost entirely protein, and a protein shortage results in decreased albumen quality, yolk, and consequently, egg size. However, it was not possible to estimate the methionine + cystine requirement from internal and external egg quality parameters, corroborating the results found by Scottá et al.[19].

According to Leeson and Summers[31], amino acids are essential components of eggs and constitute the protein molecules present in albumin and yolk. The same authors point out that the egg contains about 12% of crude protein, with 55% being present in the albumen, 42% in the yolk, and 3% in the shell. However, this chemical composition is quite stable and is difficult to modify nutritionally as the constituents are secreted by the oviduct epithelial cells.

5. Conclusion

In conclusion, Japanese quails in the laying phase require 0.90% of digestible methionine + cystine for maximum performance in egg mass, egg weight and laying rate. This is equivalent to the digestible methionine + cystine: lysine ratio as 0.83 and consumption of 241.54 mg/bird of digestible methionine + cystine/day.

Conflict of interests

The authors declare that they have no conflict of interest.

Author contributions

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