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Nutritional requirements of digestible methionine+cystine for laying Japanese quails

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ABSTRACT. The objective of the present research is to determine optimal levels of digestible methionine+cystine to maximize the laying percentage and to improve the food conversion per egg mass of Japanese quails (*Coturnix coturnix japonica*). The experimental period was divided into three evaluation cycles, using 175 Japanese quails in the laying phase, with an initial age of 60 days, distributed in a completely randomized design, with five treatments and five replications. The treatments consisted of five levels of methionine+digestible cystine (0.742; 0.842; 0.942; 1.042; 1.142%). The performance variables measured were: daily feed consumption, average egg weight, laying percentage and feed conversion per egg mass. For the three evaluation cycles there was no difference in daily feed consumption and average egg weight, while the laying percentage and feed conversion per egg mass showed a quadratic behavior with a peak egg laying percentage of 97.81% and minimum feed conversion per egg mass of 2.39. Level of methionine+cystine necessary to obtain a peak in laying percentage is 0.942%, which provides better feed conversion rates for birds, with methionine+cystine consumption estimated between 253 mg/bird/day and ratio methionine+cystine/lysine of 0.836 %.

Keywords: Feed conversion; laying percentage; sulfur amino acids; zootechnical performance.

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Introduction

The Japanese quail (*Coturnix coturnix japonica*) is a bird considered easy to manage, low maintenance cost, and good productive performance (Khosravi et al., 2016; Ray et al., 2014). Quails require more protein and less calcium in their diet when compared to chickens and layers, in addition to better digesting amino acids and fibrous foods, making better use of the energy content they hold (Fouad et al., 2018).

Formulations for Japanese quail feed are based on the concept of crude protein (CP), however, many diets result in an amino acid content that is high or low in quantity than is required, thus causing changes in the production of these birds. Therefore, when formulating diets, the use of synthetic amino acids is necessary when the crude protein content is lower than those recommended in the food composition tables and nutritional requirements for poultry and pigs (Rostagno et al., 2017).

Amino acids have several metabolic functions in addition to being necessary for protein synthesis and essential for animal development and maintenance of biological processes (Rodrigues et al., 2023). Methionine and cystine are sulfur amino acids considered to be the first limiting amino acids in poultry diets (Khosravi et al., 2016; Rodrigues et al., 2023).

Methionine is considered the first limiting amino acid, being essential when the feed ingredients are not sufficient to meet the birds' maintenance and growth needs, while cysteine is found in the ingredients and is synthesized in the animal's body from methionine, classifying it into a non-essential amino acid (Khosravi et al., 2016).

The requirement for methionine and cystine has been widely studied in meat quails, as reported by: Castro et al. (2016); Castro et al. (2018); Perine et al. (2023). However, for quails in the laying phase, studies calling for methionine + cystine are rare (Rodrigues et al., 2023; Costa et al., 2009).

Therefore, the objective of this study was to determine optimal levels of digestible methionine+cystine to maximize the laying percentage and to improve the food conversion of Japanese quails (*Coturnix coturnix* japonica) in the laying phase, from 60 to 145 days of age.

Materials and Methods

Study area

The research was conducted in the experimental aviary of the production site in the Ivan Souto settlement, in the aviary of Fazenda São João, located in the municipality of Serra Talhada-PE, in the micro-region of the Sertão do Pajeú, mesoregion of the Sertão de Pernambuco, from March to August 2023, totaling 145 days, under license number 5372030323 (ID 001412) of the Ethics Committee on the Use of Animals of the Federal Rural University of Pernambuco.

Experimental design

During the experimental period, three evaluation cycles were carried out, in each cycle 175 Japanese quails (*Coturnix coturnix japonica*) of the Fujikura lineage were used in the laying phase, with 60 days of life, distributed in a completely randomized design, with five treatments and five repetitions, totaling 25 experimental units.

The birds were housed in metal cages made of galvanized wire measuring 1.0 m length x 0.25 m width x 0.20 m high, distributed in batteries each containing three plots, with seven birds per plot. Each plot was equipped with a nipple drinker, trough feeder and egg collection spout.

The treatments consisted of five increasing levels of methionine+digestible cystine (0.742; 0.842; 0.942; 1.042; 1.142%) supplemented by a blend of organic minerals, composed of: copper amino acid chelate; selenium amino acid complex; iron amino acid chelate; calcium iodate; inactivated and dehydrated sugar cane yeast; manganese amino acid chelate and zinc amino acid chelate, in the following mineral concentrations (minimum copper 7,000 mg Kg^{-1}; minimum iron 30 g Kg^{-1}; minimum iodine 1,200 mg Kg^{-1}; minimum manganese 50 g Kg^{-1}; minimum selenium 200 mg Kg^{-1}; minimum zinc 45 g Kg^{-1}). These supplements were included in the diet at the rate of 1 kg of the blend per ton of feed.

The formulation of the rations was carried out following the recommendations proposed by Rostagno et al. (2017), with a content of 18.92% of crude protein and 2,800 kcal kg^{-1} of metabolized energy, with these diets being isoenergetic and isolysinic, differing from each other in terms of digestible methionine supplementation for the composition of different levels of digestible methionine+cystine and are presented in Table 1.

Table 1. Composition of experimental diets depending on digestible methionine+cystine levels.

Ingredients (g kg^{-1})	Levels of methionine+cystine (%)				
	0.742	0.842	0.942	1.042	1.142
Corn (kg)	59.239	61.169	61.130	61.091	61.052
Soybean meal (45%)	26.673	27.420	27.240	27.060	26.877
Wheat bran	4.748	2.144	2.249	2.354	2.457
Calcitic limestone	6.816	6.800	6.801	6.802	6.801
Dicalcium phosphate	1.065	1.091	1.091	1.091	1.092
DL-methionine (99%)	0.243	0.334	0.438	0.542	0.646
L-lysine HCl (78%)	0.457	0.414	0.419	0.425	0.431
NaCl	0.276	0.276	0.276	0.276	0.276
L-threonine (98%)	0.215	0.105	0.108	0.111	0.057
L-tryptophan	0.066	0.047	0.048	0.049	0.050
[1]Premix mineral/vitamin	0.100	0.100	0.100	0.100	0.100
Blend of organic minerals	0.100	0.100	0.100	0.100	0.100
Calculated Composition (%)					
Crude protein (%)	18.92	18.92	18.92	18.92	18.92
Metabolizable energy (kcal kg^{-1})	2800	2800	2800	2800	2800
Calcium (%)	2.990	2.990	2.990	2.990	2.990
Phosphorus available (%)	0.309	0.309	0.309	0.309	0.309
Digestible lysine (%)	1.149	1.149	1.149	1.149	1.149
Digestible methionine (%)	0.490	0.590	0.690	0.790	0.890
Digestible methionine+cystine (%)	0.742	0.842	0.942	1.042	1.142
Digestible threonine (%)	0.701	0.701	0.701	0.701	0.701
Digestible tryptophan (%)	0.241	0.241	0.241	0.241	0.241
Sodium (%)	0.147	0.147	0.147	0.147	0.147
Fat (%)	3.942	2.567	2.566	2.566	2.566
Linoleic acid	1.310	1.384	1.384	1.383	1.383

[1]Premix mineral vitamin kg^{-1}: Folic Acid 106.00 mg; Pantothenic 2,490 mg; Antifungal 5,000 mg; Antioxidant 200 mg; Biotin 21mg; Coccidiostatic 15,000 mg; Choline 118,750 mg; Vitamin K3 525.20 mg; niacin 7,840 mg; Pyridoxine 210 mg; Riboflavina 1,660 mg; Thiamine 360 mg; Vitamin A 2,090,000 UI; Vitamin B12 123,750 mcg; Vitamin D3 525,000 UI; Vitamin E 4,175 mg. Cu 2,000 mg; I 190 mg; Mn 18,750 mg; Se 75 mg; Zn 12,500 mg.

Management was carried out in accordance with the recommendations of the Fujikura company, holder of the genetic material, and from the forty-fifth day of life the birds were subjected to a 17-hour light program, controlled by an automatic watch (timer) and offered experimental diets according to the treatments. During the experimental period, sanitary control was maintained, with the removal of manure from the trays of each cage.

Variables analyzed

The variables of performance measured were: average egg production per bird day^{-1} for each treatment (%), feed consumption (g bird^{-1} day^{-1}), egg weight (g), total egg mass per treatment (g of egg bird^{-1} day^{-1}) and feed conversion per egg mass (g of feed per egg mass).

The feed, provided daily, totaled 200 g per plot, with an estimated consumption of approximately 28 g for each quail. The birds were fed in two periods, with 100 g in the morning and 100 g in the late afternoon, with leftovers being measured daily to calculate the daily feed consumption given by:

$$\text{DFC} = \text{amount of feed consumed on the day} - \text{amount of feed left over on the day}$$

Feed conversion per egg mass (FC) was determined by dividing the total feed consumed by the weight of eggs produced, expressed in grams of feed per gram of egg produced.

$$\text{FC} = \frac{\text{daily feed consumption}}{\text{egg mass}}$$

With the total weight and number of eggs per plot, the average egg weight (AEW, g) of the plots was calculated.

$$\text{AEW} = \frac{\text{total weight of eggs}}{\text{number of eggs per plot}}$$

Egg collection was carried out daily, in the morning and afternoon, the laying percentage (LP) was calculated by the number of eggs produced (whole, broken, cracked and deformed eggs) divided by the total number of eggs produced from all viable birds of each installment, multiplied by 100.

$$\text{LP} = \frac{\text{number of eggs produced}}{\text{number of eggs produced from birds on the plot}} \text{ x } 100$$

Statistical analysis

An analysis of variance was used to compare the dependent variables (AEW, LP, DFC, FC) with different levels of methionine+cystine in the quails' diet, after which pairwise differences were detected with the Tukey test at the 5% level.

For the dependent variables that showed differences in relation to the diet, a quadratic regression model was adjusted and defined by:

$$\text{Y} = \text{aX}^2 + \text{bX} + \text{c} + \varepsilon$$

where Y is the dependent variable (AEW, LP, DFC, FC), X is the independent variable (levels of methionine+cystine in the quails' diet), ε is the random error that presents a normal distribution with a mean of zero and constant variance $\sigma^2 > 0$; a, b, and c were the model parameters to be estimated (Lucena et al., 2023).

Results and discussion

In the first evaluation cycle, it was observed that there was no change in the birds' daily feed consumption in relation to the different levels of methionine + cystine in the birds' diets (p-value = 0.405), (Table 2). The same can be observed for the average egg weight (AEW) (p-value = 0.586), (Table 2).

Table 2. Average performance of Japanese quails in laying as a function of digestible methionine + cystine levels in the first cycle evaluation (60 to 88 days).

	Methionine+cystine levels (%) (Mean±SD)					p-valor
	0.742	0.842	0.942	1.042	1.142	
DFC	24.77±1.42a	24.51±1.08a	25.62±0.97a	24.56±0.40a	25.00±0.71a	0.405
AEW	11.22±0.28a	10.94±0.32a	10.96±0.55a	10.83±0.50a	10.90±0.26a	0.586
LP	84.98±4.75c	94.69±3.03a	92.44±3.82a	87.75±4.89b	86.32±6.76bc	< 0.0001
FC	2.63±0.12ab	2.41±0.10c	2.34±0.24d	2.59±0.17b	2.66±0.22a	< 0.0001

identical letters do not differ in the Tukey test. DFC- daily feed consumption; AEW- average egg weight; LP- laying percentage; FC- feed conversion per egg mass.

The birds showed a higher laying percentage (94.69%) at the 0.842 level of methionine plus cystine and a lower percentage at the 0.742% level of methionine + cystine (p-value < 0.0001), Table 2. The birds maximized their laying percentage at 92.8% when using the optimal level of 0.93% methionine + cystine, (Figure 1).

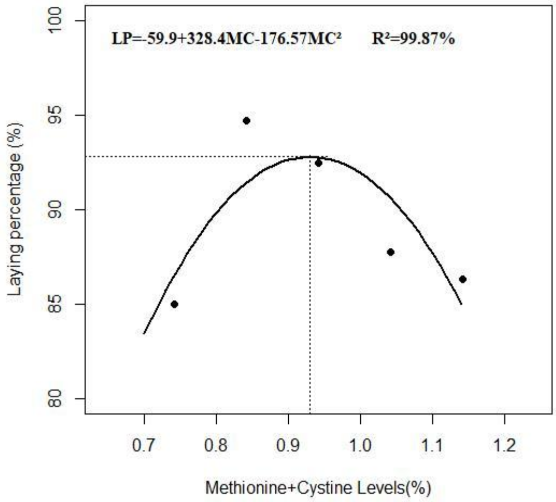

Figure 1. Laying percentage of quails in relation to different levels of methionine + cystine in the first cycle evaluation (60 to 88 days).

The birds showed lower feed conversion (2.34) at the level of 0.942% of methionine + cystine and higher conversion (2.66) at the level of 1.142% of methionine + cystine (p-value < 0.0001), (Table 2). They minimized their feed conversion by 2.39 when using the optimal level of 0.923% methionine + cystine, (Figure 2).

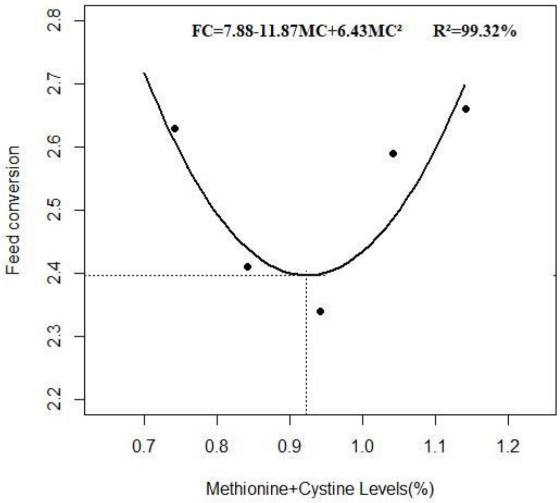

Figure 2. Feed conservation mass per egg mass of quails in relation to different levels of methionine + cystine in the first cycle evaluation (60 to 88 days).

In the second evaluation cycle, it was verified that there was no difference in daily feed consumption (p-value = 0.372) as well as in the average egg weight (p-value = 0.476) in relation to the different levels of methionine + cystine in the birds' diets, (Table 3).

Table 3. Average performance of Japanese quails in laying as a function of digestible methionine + cystine levels in the evaluation second cycle (89 to 117 days).

	Methionine+cystine levels (%) (Mean±SD)					p-valor
	0.742	0.842	0.942	1.042	1.142	
DFC	26.01±1.99a	26.68±0.95a	24.88±1.90a	25.30±1.15a	26.06±1.04a	0.372
AEW	11.08±0.55a	10.74±0.47a	11.65±0.78a	10.91±0.35a	11.40±0.45a	0.476
LP	93.98±4.35b	97.16±2.68a	97.26±3.17a	96.00±5.87ab	90.30±4.39c	< 0.0001
FC	2.62±0.17a	2.57±0.29ab	2.46±0.15c	2.49±0.13c	2.53±0.07b	< 0.0001

identical letters do not differ in the Tukey test. DFC- daily feed consumption; AEW- average egg weight; LP- laying percentage; FC- feed conversion per egg mass.

The birds showed the highest laying percentage at levels of 0.842 and 0.942% of methionine + cystine in the diets, while the lowest laying percentage was related to the level of 1.142%, (Table 3). The optimal level of 0.911% of methionine + cystine maximized the laying percentage of quail at 97.81%, (Figure 3).

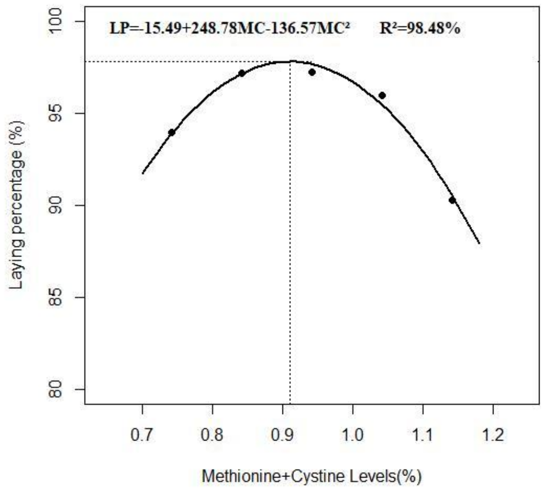

Figure 3. Laying percentage of quails in relation to different levels of methionine + cystine in the second cycle evaluation (89 to 117 days).

The birds showed lower feed conversions at levels of 0.942% and 1.042% of methionine plus cystine and higher conversion (2.62) at the level of 0.742% of methionine + cystine (p-value < 0.0001), (Table 3). Quails minimized its feed conversion at 2.48 when using the optimal level of 0.998% methionine + cystine, (Figure 4).

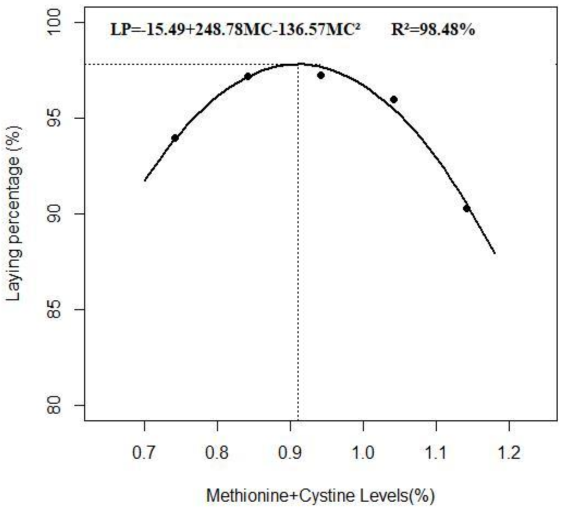

Figure 4. Feed conservation per egg mass of quails in relation to different levels of methionine + cystine in the second cycle evaluation (89 to 117 days).

In the third evaluation cycle (birds aged 118 to 145 days) it was verified that there was no difference in daily feed consumption (p-value = 0.684) and average egg weight (p-value = 0.532) in relation to the different levels of methionine + cystine in birds' diets, (Table 4).

Table 4. Average performance of Japanese quails in laying as a function of digestible methionine + cystine levels in the third cycle evaluation (118 to 145 days).

	Methionine+cystine levels (%) (Mean±SD)					p-valor
	0.742	0.842	0.942	1.042	1.142	
DFC	25.92±2.76a	26.28±1.45a	25.98±1.43a	25.72±1.34a	26.22±1.29a	0.684
AEW	11.38±0.19a	10.74±0.35a	10.90±0.29a	11.20±0.18a	11.04±0.49a	0.532
LP	92.93±7.43c	95.03±4.13b	98.67±1.46a	96.34±6.08b	92.94±2.76c	< 0.0001
FC	2.73±0.22a	2.60±0.49b	2.41±0.19c	2.48±0.32c	2.64±0.27b	< 0.0001

identical letters do not differ in the Tukey test. DFC- daily feed consumption; AEW- average egg weight; LP- laying percentage; FC- feed conversion per egg mass.

In the third evaluation cycle, the quails presented the highest laying percentage (98.67%) when using the level of 0.942% of methionine plus cystine in the diet, while the lowest laying percentage was attributed to the birds that received 0.742 and 1.142% of methionine + cystine in their diet, respectively, (Table 4). The quails maximized their laying percentage by 97.61% when using the optimal level of 0.948% of methionine + cystine in their diet, (Figure 5).

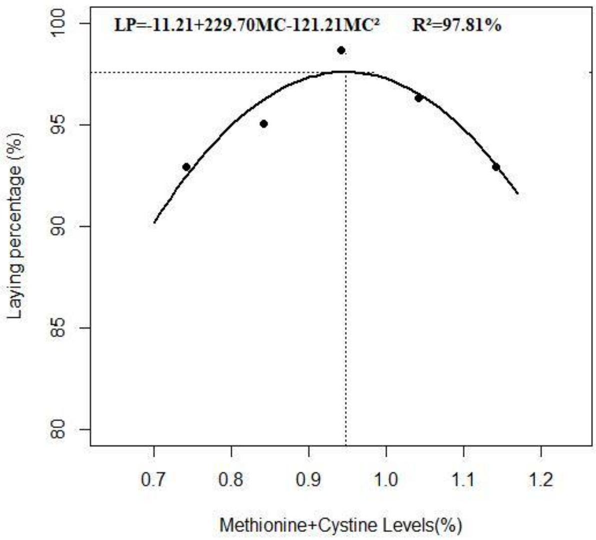

Figure 5. Laying percentage of quails in relation to different levels of methionine + cystine in the third cycle evaluation (118 to 145 days).

The birds showed lower feed conversions at levels of 0.942% and 1.042% of methionine + cystine and higher conversion (2.73) at the level of 0.742% of methionine + cystine (p-value < 0.0001), (Table 4). Quails minimized its feed conversion at 2.45 when using the optimal level of 0.967% methionine + cystine, (Figure 6).

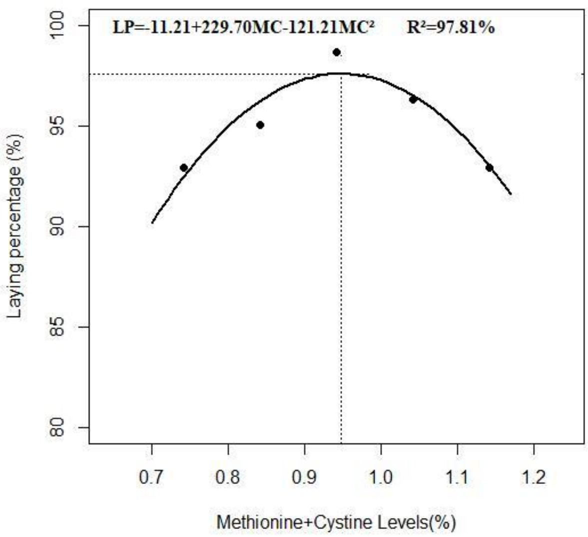

Figure 6. Feed conservation per egg mass of quails in relation to different levels of methionine + cystine in the third cycle evaluation (118 to 145 days).

The food consumption of the birds was not influenced by the different levels of methionine + cystine in the three evaluation cycles. It possibly occurred because the formulated diets met nutritional requirements (with the blend of organic minerals), without the quails increase their consumption preventing the methionine to become toxic in their organism. Amino acids possess several metabolic functions; however, their main function is related to the formation of body proteins (muscle mass), making their supplementation an efficient tool for weight gain and food conversion (Perine et al., 2023).

The laying percentage in the three evaluation cycles varied between 92.4% and 98.67%, when using methionine + cystine levels between 0.842% and 0.942%, similar results were reported by Rodrigues et al.

(2023) who determined the level of 0.903% of methionine+cystine as being capable of improving the laying rate by 90.45%. This result can be explained because the methionine is the amino acid that starts protein synthesis and furthermore, one of its major paths of action is to act in egg production, influencing egg weight, number and percentage (Costa et al., 2009).

Recent research suggests that levels above 0.77% result in better performance (Khosravi et al., 2016; Parvin et al., 2010; Rostagno et al., 2017). In the last 30 years, there has been a genetic improvement of this species, transforming quails into more productive birds and, consequently, with greater nutritional needs to direct the deposition of tissues which improve its efficiency. The synthesis of non-essential amino acids has an energy demand and the correct balance between essential and non-essential amino acids can provide greater efficiency in the use of dietary nitrogen (Van Milgen & Dourmad, 2015).

Diets in the laying phase with 0.942% methionine + cystine were sufficient to increase productivity in quantity (number of eggs), which are directly related to feed conversion. 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 mechanisms of action is to act on egg production, influencing weight and laying rate. (Costa et al., 2009).

On the three evaluation cycles it was observed that the feed conversion was smaller when using methionine + cystine levels between 0.842 and 0.942%. That can be explained as feed conversion is directly linked to the laying percentage, egg weight and consumption of feed that presented the best results when using methionine plus cystine levels of 0.942%. The influence of such levels of methionine + cystine possibly promoted the amino acid imbalance resulting in changes on the plasma profile of the birds. Such changes in the plasma profile activate appetite regulatory mechanisms, which cause an increase in food intake.

Rodrigues et al. (2023) detected an optimal feed conversion with an index of 2.67 when the determined optimal level of methionine + digestible cystine in the diets was 0.88%, while Perine et al. (2023) found an optimal level of 2.8 for feed conversion when using 0.82% methionine + digestible cystine. Rodrigues et al. (2023) and Perrine et al. (2023) observed a quadratic effect on the feed conversion of birds, corroborating the results presented in this study. These results were obtained due to a greater use of nutrients in the feed with an adequate balance of amino acids.

Conclusion

The level of methionine+cystine necessary to provide better laying rates and average egg weight is 0.942%, which provides better feed conversion rates for birds, with methionine+cystine consumption estimated between 253 mg bird^{-1} day^{-1} and ratio methionine+cystine/lysine of 0.836%.

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