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Relationship of methionine plus cystine with lysine in diets for laying Japanese quails

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ABSTRACT - The objective of this work was to evaluate the relationships of methionine plus cystine with lysine for Japanese quails in the laying phase. Thus, it was used 480 birds at initial age of 65 days, distributed in randomized block design with 6 treatments, 10 replicates and 8 birds per experimental unit. The treatments consisted of basal diet deficient in methionine plus cystine (0.65%), with 19.6% of crude protein and 2800 kcal of metabolizable energy/kg of ration, supplemented with six levels of DL-methionine 98% (0.116; 0.167; 0.218; 0.269; 0.320 and 0.371%), corresponding to methionine plus cystine with lysine ratio of 0.65, 0.70, 0.75, 0.80, 0.85 and 0.90, and the digestible lysine set at a suboptimal level of 1.00. The parameters studied were: feed intake, egg production per hen day, egg production per housed hen, commercial egg production, egg weight, egg mass, feed conversion per egg mass, feed conversion per dozen eggs, viability of birds, weight of the egg components (yolk, albumen and shell), percentage of components of eggs, diameter and height of the eggs and specific gravity. It was observed an increasing linear effect for feed intake, egg diameter, shell weight and percentage of yolk. For eggshell percentage, it was observed a decreasing linear effect. For the weight of the eggs, the best adjustment was obtained by using the Linear Response Plateau model. A quadratic effect was observed for egg mass, feed conversion per egg mass, egg production per hen day, egg production per housed hen, yolk weight, albumen weight and height of the eggs. The relationship of methionine plus cystine with lysine of 0.84 which corresponds to the consumption of 221.0 mg/bird/day of methionine + cystine provides better performance and satisfactory quality of Japanese quail eggs.

Key Words: *Coturnix coturnix japonica*, digestible amino acid, egg production, ideal protein, performance

Relação metionina mais cistina com lisina em dietas para codornas japonesas em postura

RESUMO - Com o objetivo de avaliar o efeito de relações entre metionina mais cistina com lisina para codornas japonesas na fase de postura, 480 aves com idade inicial de 65 dias foram distribuídas em delineamento em blocos casualisados com 6 tratamentos, 10 repetições e 8 aves por unidade experimental. Os tratamentos consistiram de ração basal deficiente em metionina mais cistina (0,65%), com 19,6% de proteína bruta e 2800 kcal de energia metabolizável/kg de ração, suplementada com seis níveis de DL-metionina 98% (0,116; 0,167; 0,218; 0,269; 0,320 e 0,371%), correspondendo à relação metionina mais cistina com lisina de 0,65; 0,70; 0,75; 0,80; 0,85 e 0,90, sendo a lisina digestível fixada no nível sub ótimo de 1,00. Os parâmetros estudados foram: consumo de ração, produção de ovos por ave dia, produção de ovos por ave alojada, produção de ovos comercializáveis, peso do ovo, massa de ovos, conversão alimentar por massa de ovos, conversão alimentar por dúzia de ovos, viabilidade das aves, peso dos componentes dos ovos (gema, albúmen e casca), porcentagem dos componentes dos ovos, diâmetro e altura dos ovos e gravidade específica. Observou-se efeito linear crescente para consumo de ração, diâmetro dos ovos, peso da casca e porcentagem de gema. Para porcentagem de casca foi observado efeito linear decrescente. Para o peso dos ovos o melhor ajuste foi obtido com a utilização do modelo Linear Response Plateau. Foi verificado efeito quadrático para a massa de ovos, conversão alimentar por massa de ovos, produção de ovos por ave dia, produção de ovos por ave alojada, peso da gema, peso do albúmen e altura dos ovos. A relação metionina mais cistina com lisina de 0,84 que corresponde ao consumo de 221,0 mg/ave/dia de metionina + cistina proporciona melhor desempenho e satisfatória qualidade de ovo de codornas japonesas.

Palavras-chave: aminoácido digestível, *Coturnix coturnix japonica*, desempenho, produção de ovos, proteína ideal

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Introduction

In 2007, the breeding of quails in Brazil was estimated in 7.586 million birds, 5.3% higher than in 2006. With the growth of the flock, it was observed that egg production increased by 5.9% in 2007, surpassing 131 million dozen of eggs (IBGE, 2007). The physical growth of the herds and the increase in egg production showed a gain in productivity resulting from better technology involved in the genetics of quails and the cultures in general. Quails became heavier, more productive with larger eggs and with higher resistance (Oliveira, 2007).

Methionine is the first limiting amino acid in diets based on corn and soybean meal, and its deficiency is corrected by supplementation with synthetic methionine. Waldroup & Hellwig (1995) reported that differences in the determination of methionine requirement for laying hens over the years is quite understandable due to major changes in genetics, nutrition and management which the birds are subjected to, besides the effects of age, type of diet and environmental conditions.

Methionine is the main donor of methyl group (S-adenosylmethionine) for the various metabolic reactions and it directly participates in protein synthesis (Leeson & Summers, 2001). It also serves as an alternative source of cystine in a non-reversible process, playing a special role in the structure of many proteins (immunoglobulins, hormone insulin) and linking the polypeptide chains by disulfide bonds (Lenningher, 1996). In laying birds, methionine interferes significantly in the production (Sá et al., 2007) and egg weight (Narváez-Solarte et al., 2005; Pavan et al., 2005).

The determination of animal requirements of amino acids based on ideal protein concept allows reducing the levels of dietary crude protein with consequent industrial amino acids supplementation in crystalline form, generally maximizing protein utilization, reducing safety margins and consequently reducing the cost and emission of pollutants into the environment (Moura, 2004).

The objective of this work was to establish the best relationship of methionine plus cystine with lysine for laying Japanese quails.

Material and Methods

The experiment was conducted in the Setor de avicultura do Departamento de Zootecnia da Universidade Federal de Viçosa, at the Universidade Federal de Viçosa-MG, from October to December, 2008 with a duration of 63 days, divided into three 21-day periods. It was used 480 quails of Japanese subspecies *(Coturnix coturnix japonica)* at 65 days of age, distributed in a complete randomized block design. The treatments consisted of six ratios of methionine plus cystine or lysine, with ten replicates and eight birds per experimental unit.

It was formulated a basal diet (Table 1), supplemented with six levels of DL-methionine (0.116, 0.167, 0.218, 0.269, 0.320 and 0.371%), replacing the glutamic acid in protein equivalents, corresponding to the ratio of methionine plus cystine with lysine in the diets of 0.65, 0.70, 0.75, 0.80, 0.85 and 0.90. The differences from the balancing to the protein equivalent of methionine plus cystine and glutamic acid in different ratios of methionine plus cystine with lysine in the evaluation were offset by the starch.

The relationships of digestible threonine and tryptophan with lysine adopted in this research were the same used by Umigi et al. (2008) and Pinheiro et al. (2008), respectively. As the other relationships of amino acids were not determined on the digestible basis, they were seen maintaining the relationship total amino acid with lysine as recommended in the NRC (1994). The energy level of diet used was 2,800 kcal/kg as determined by Moura et al. (2008); the levels of calcium and phosphorus were corrected for energy density of the diet according to the same author. The other requirements were met in accordance with those recommended by NRC (1994).

The composition and nutritional value of ingredients used to formulate the diet were calculated according to Rostagno et al. (2005).

The birds were housed in galvanized wire cages equipped with feeders and nipple drinkers. Each cage provided a space of 106 cm^2/bird.

The food and water were provided ad libitum throughout the experimental period.

The daily management was to collect and to count the eggs (including eggs broken, cracked, with a soft shell and shelled), to record the temperatures (°C) and relative humidity in the experimental aisle once a day, at 4 p.m. by using thermometers and to record maximum and minimum dry and wet bulb, positioned at a central point at the level of the birds. The lighting program consisted of providing 17 hours of light per day, and it was controlled by an automatic watch (timer) that allowed the lights turn on and off automatically overnight, according to the procedure adopted on commercial farms.

At the end of experimental period (63 days), the amount of feed consumed (g/bird/day) in each experimental unit was determined. To do so, food scraps and waste products were weighed and subtracted from the amount of feed supplied during the experimental period. The number of

Table 1 - Percentage and calculated compositions in the experimental diets, as fed basis

Ingredients	T1	T2	T3	T4	T5	T6
Corn	59.277	59.277	59.277	59.277	59.277	59.277
Soybean meal (45%)	30.634	30.634	30.634	30.634	30.634	30.634
Soybean oil	0.670	0.670	0.670	0.670	0.670	0.670
Limestone	6.633	6.633	6.633	6.633	6.633	6.633
Dicalcium phosphate	1.067	1.067	1.067	1.067	1.067	1.067
Salt	0.320	0.320	0.320	0.320	0.320	0.320
DL-Methionine (99%)	0.116	0.167	0.218	0.269	0.320	0.371
L-Lysine HCL (79%)	0.120	0.120	0.120	0.120	0.120	0.120
L-Isoleucine (99%)	0.187	0.187	0.187	0.187	0.187	0.187
L- Valine (99%)	0.133	0.133	0.133	0.133	0.133	0.133
L- Arginine (99%)	0.150	0.150	0.150	0.150	0.150	0.150
L- Tryptophan (99%)	0,033	0.033	0.033	0.033	0.033	0.033
Starch	0.100	0.103	0.106	0.108	0.110	0.112
Glutamic acid	0.300	0.246	0.192	0.139	0.086	0.033
Choline chloride (60%)	0.100	0.100	0.100	0.100	0.100	0.100
Vitamin supplement[1]	0.100	0.100	0.100	0.100	0.100	0.100
Mineral supplement[2]	0.050	0.050	0.050	0.050	0.050	0.050
Antioxidant[3]	0.010	0.010	0.010	0.010	0.010	0.010
Calculated composition						
Metabolizable energy (kcal/kg)	2.800	2.800	2.800	2.800	2.800	2.800
Crude protein (%)	19.66	19.66	19.66	19.66	19.66	19.66
Digestible lysine (%)	1.000	1.000	1.000	1.000	1.000	1.000
Total lysine (%)	1.080	1.080	1.080	1.080	1.080	1.080
Methionine + digestible cystine (%)	0.650	0.700	0.750	0.800	0.850	0.900
Digestible methionine (%)	0.390	0.440	0.490	0.540	0.590	0.680
Digestible threonine (%)	0.640	0.640	0.640	0.640	0.640	0.640
Digestible tryptophan (%)	0.240	0.240	0.240	0.240	0.240	0.240
Total isoleucine (%)	1.000	1.000	1.000	1.000	1.000	1.000
Total valine (%)	1.030	1.030	1.030	1.030	1.030	1.030
Total arginine (%)	1.390	1.390	1.390	1.390	1.390	1.390
Calcium (%)	2.900	2.900	2.900	2.900	2.900	2.900
Available phosphorus (%)	0.300	0.300	0.300	0.300	0.300	0.300
Sodium (%)	0.140	0.140	0.140	0.140	0.140	0.140
Crude fiber (%)	2.680	2.680	2.680	2.680	2.680	2.680

[1] Composition/kg of product: A vit. - 12,000,000 U.I.; D_3 vit. - 3,600,000 U.I.; E vit. - 3,500 U.I.; B_1vit - 2,500 mg; B_2 vit. - 8,000 mg; B_6 vit - 5,000 mg; antothenic acid - 12,000 mg; biotin - 200 mg; K vit.- 3,000 mg; folic acid - 1,500 mg; nicotinic acid - 40,000 mg; B_{12}vit. - 20,000 mg; Selenium - 150 mg; vehicle q.s.p. - 1,000 g.
[2] Composition/kg of product: Mn - 160 g; Fe - 100 g; Zn - 100 g; Cu - 20 g; Co - 2 g; I - 2 g; vehicle q.s.p.: 1,000 g.
[3] Butylated hydroxy toluene - BHT (99%).

dead birds was subtracted from the total number of birds in each experimental unit, which allowed obtaining the correct consumption per bird.

The egg production was expressed as a percentage of bird day (egg/bird/day) and birds were housed at the beginning of the experiment (egg/housed bird). These data were obtained by computing the daily number of eggs produced, including the broken, the cracked and abnormal (eggs with soft shell and shelled) eggs. The average number of marketable eggs during the experimental period was calculated by withdrawing the broken, cracked and abnormal eggs from the total number of eggs produced.

To obtain the specific gravity of eggs at 16, 17 and 18 days from each of the three experimental periods, all intact eggs were immersed and evaluated in NaCl solutions with densities from 1.055 to 1.100 g/cm^3, with ranges of 0.005 g/cm^3 between them. The density of the egg was measured by means of a hydrometer.

All intact eggs produced in each replicate were weighed on a 0.01 g precision balance on days 19, 20 and 21 of each of the three experimental periods to obtain the average weight of eggs. After weighing, it was randomly selected four eggs from each repetition to quantify the eggs. Eggs selected for each repetition and each day were individually weighed on a 0.001 g precision balance. After weighing, they were identified and subsequently broken. The yolk of each egg was weighed and recorded, its shell was washed, and air dried for further weighing. The weight of albumen was obtained by the difference between the egg weight and yolk weight plus the weight of the shell.

The average weight of eggs was multiplied by the total number of eggs produced in the period, resulting in the total mass of eggs per period. Total mass of eggs was divided by the total number of birds of the period and by the number of days in the period, and finally expressed in grams of egg/hen/day. The conversion per dozen eggs, expressed as the total feed intake in kilograms divided by the dozen eggs produced (kg/ dozen), and conversion by egg mass, which was obtained by the feed intake in kilograms divided by the mass of eggs produced in kilograms (kg/kg) were evaluated.

The parameters were subjected to analysis of variance and regression, according to SAEG (UFV, 2007). Estimates for determining the best relationship of methionine plus cystine with lysine was calculated by linear and square regression analysis, as the best fit obtained for each parameter, considering the biological behavior of birds.

Results and Discussion

In the mature phase, the thermal comfort range of quail is from 18 to 22°C, and the relative humidity range from 65 to 70% (Oliveira, 2004). Thus, according to the values recorded for the dry bulb thermometer (Table 2), it is possible to observe that the quails were in mild conditions of heat stress in part of the experiment.

Relationships of methionine plus cystine with lysine had a linear effect on feed intake according to the equation $\hat{Y} = 23.5978 + 3.49051x$; $R^2 = 0.73$ (Table 3). Belo et al. (2000), working with five levels of DL-methionine (0.283, 0.355, 0.428, 0.501 and 0.573) and Pinto et al. (2003), determining the relationship methionine plus cystine with lysine by using the ratios 0.60, 0.65, 0.70, 0.75, 0.80, 0.85, found an increase in consumption with increasing levels of sulfur amino acids in the diet. However, Murakami et al. (1994), evaluating the levels of 0.35, 0.45, 0.55 and 0.65% methionine plus cystine in the diet, Stringhini et al. (1998) by checking the effects of the combination of three levels of energy (2,700, 2,850 and 3,000 kcal/kg) with two methionine (0.45 and 0.50%) and Garcia et al. (2005), evaluating three levels of CP (16.0, 18.0 and 20.0%), three levels of methionine plus cystine (0.700, 0.875 and 1.050%) and two levels of lysine (1.10% and 1.375%) found no significant effect on the levels of sulfur amino acids on feed intake. In this study, the increase in feed intake with a consequent increase in the consumption of sulfur amino acids, allowed to infer that the relationships of methionine plus cystine with lysine used were not sufficient to produce amino acid imbalance that resulted in changing the plasma profile of the animal by activating the mechanisms of appetite regulation, as described by Harper (1970).

It was observed a quadratic effect of relationships of methionine plus cystine with lysine for the parameters of eggs per bird day according to the equation $\hat{Y} = -20.8414 + 293.821x - 185.239x^2$; $R^2 = 0.84$ and for egg production per hen housed according to the equation $\hat{Y} = -79.9764 + 440.843x - 277.495x^2$; $R^2 = 0.91$, both being maximized in the ratio of 0.79. Murakami et al. (1994) and Pinto et al. (2003) found a quadratic effect of dietary sulfur amino acid levels on egg production per bird day, and found the level of 0.45% and ration of 0.71 respectively. However, Garcia et al. (2005) found no effect of methionine plus digestible cystine on this parameter. In literature, there are few scientific studies determining the production of eggs per quail housed. This is an important parameter to be observed because the mortality of these birds during the laying period is high when compared to the mortality of laying hens.

Table 2 - Values of temperature and relative humidity (RH), recorded in the experimental shed

Schedule	Air temperature (°C)			RH (%)
	Maximum	Minimum	Dry bulb	
8:00 a.m.	-	-	26.6 ± 1.2	76.8 ± 8.5
4:00 p.m.	26.7 ± 3.1	19.9 ± 3.2	25.4 ± 2.3	67.4 ± 10.7

Table 3 - Relationship of methionine plus cystine with lysine on the performance parameters

Parameter	Relationship of methionine plus cystine with lysine						P Value	CV (%)
	0.65	0.70	0.75	0.80	0.85	0.90		
Feed intake (g)	25.78	26.23	25.94	26.57	26.69	26.59	P<0.02	3.56
Eggs/day hen (%)	91.83	94.52	94.71	95.33	96.05	93.13	P<0.02	3.93
Eggs/housed hen (%)	88.97	93.55	94.21	94.33	95.04	91.81	P<0.02	5.54
Commercial egg (%)	96.90	97.60	97.90	97.00	97.90	98.00	NS	2.01
Eggs weight (g)	10.66	11.13	11.32	11.44	11.70	11.71	P<0.01	2.12
Egg mass (g/bird/day)	9.79	10.52	10.72	10.91	11.25	10.91	P<0.01	4.32
Feed conversion (kg/kg)	2.61	2.47	2.42	2.44	2.38	2.44	P<0.01	3.97
Feed conversion (kg/dozen)	0.33	0.33	0.33	0.34	0.33	0.34	NS	3.78
Viability (%)	92.50	98.75	98.75	97.50	98.75	97.50	NS	7.24

CV = Coefficient of variation; NS = Not significant, P>0.05.

Relationships of methionine plus cystine with lysine in the diet did not affect the quality of eggs to be marketed.

It was observed increase in egg weight by the ratio of 0.84 according to the LRP model according to the equation $\hat{Y}$ = 7.44519 + 5.09460x; R^2 = 0.90. Murakami et al. (1994), Belo et al. (2000), Pinto et al. (2003) and Souza et al. (2004) found an improvement on egg weight with the addition of sulfur amino acids in the diet. However, Garcia et al. (2005) and Stringuini et al. (1998) found no increase in egg weight with increasing levels of methionine plus cystine in the diets, although the authors noted an increase in weight of the eggs in absolute terms. The consumption of protein influences the weight of the eggs. Because hens have little ability to store protein, the level of feed intake are important to the daily intake of this nutrient in order to meet the demands of producing heavier eggs (Pesti et al., 1992). Because the amino acid methionine, whose main function is to act on egg production in terms of weight, a limit on the amount of this amino acid in the diet can lead to production of smaller eggs and therefore a lower egg mass production.

There was an effect of relationships of methionine plus cystine with lysine on egg mass, and this was quadratically improved by the ratio of 0.84 according to the equation $\hat{Y}$ = -13.1007+57.4577x -34.1269x²; R^2 = 0.95. Pinto et al. (2003) and Souza et al. (2004) also observed a quadratic effect of the inclusion of sulfur amino acids on this parameter, the relationship being found at 0.727 and 0.87% respectively. The ratio of methionine plus cystine with lysine checked for egg mass is consistent with that observed for laying and egg weight inasmuch as this parameter depends on the egg production and on the egg weight.

The feed conversion by egg mass was influenced by the relationship of sulfur amino acids with lysine, which was quadratically improved by the ratio of 0.82 according to the equation $\hat{Y}$ = 7.03755 -11.2984 +6.8757x²; R^2 = 0.91. Souza et al. (2004) found an improvement in the conversion to the level of 0.88% methionine plus cystine. Contrarily, Pinto et al. (2003) and Garcia et al. (2005) found no effect of sulfur amino acid supplementation on this parameter. However, Belo et al. (2000) observed a linear decrease in the levels of these amino acids on feed conversion by egg mass. No significant effect was observed on the relationships of methionine plus cystine with lysine on feed per dozen eggs. Garcia et al. (2005) also found no improvement in the conversion with increasing levels of sulfur amino acids in the diet.

No significant effect on relationships of methionine plus cystine with lysine was observed on the viability. In this study, the mortality rate observed was 2.7%, representing 0.30% mortality weekly. Oliveira (2007), to assess the egg production and viability of 26 flocks of a commercial production covering 400,000 Japanese quail reared in manual and automated systems, mentioned that the viability accumulated in birds at 52 weeks of age was 74.53%, corresponding to a total mortality rate of 25.47%, or an average mortality of 0.49% per week.

It was observed a quadratic effect of methionine plus cystine with lysine ratio on egg yolk weight according to the equation $\hat{Y}$ = -1.33088 +10.8794x -6. 2371x²; R^2 = 0.99, the same being maximized in the ratio of 0.87 (Table 4). However, for the percentage of yolk, it was found a linear effect according to equation $\hat{Y}$ = 26.6898+2.43429x; R^2 = 0.64. The increase in yolk weight may be related to the formation of choline from methionine, added to the phospholipids to form lipoproteins of yolk (Brumano et al., 2010). For albumen weight, according to the quadratic equation $\hat{Y}$ = -0.906051 +19.4526x -11.2643x²; R^2 = 0.96, and the relationship of 0.86, it was observed higher weight. Considering that in the magnum protein synthesis is greatest at which time albumen is secreted within a 3-h period, changes in amino acid concentration in blood as a result of dietary intake may have less of an effect on synthesis rates in the liver tissue. Rapid protein synthesis in the magnum

Table 4 - Relationship of methionine plus cystine with lysine on the parameters of egg quality

Parameter	Relationship of Methionine plus cystine with lysine						P Value	CV (%)
	0.65	0.70	0.75	0.80	0.85	0.90		
Yolk weight (g)	3.12	3.21	3.33	3.38	3.44	3.40	P<0.01	2.40
Yolk (%)	28.31	28.14	28.72	28.67	28.87	28.73	P<0.01	1.63
Albumen weight (g)	6.95	7.26	7.31	7.44	7.49	7.48	P<0.01	2.73
Albumen (%)	63.15	63.57	62.99	63.19	62.98	63.16	NS	0.81
Shell weight (g)	0.94	0.95	0.96	0.96	0.97	0.96	P<0.05	3.19
Shell (%)	8.54	8.29	8.29	8.14	8.15	8.11	P<0.01	2.91
Egg diameter (mm)	26.91	27.26	27.33	27.24	27.48	27.37	P<0.05	1.47
Egg height (mm)	31.31	31.86	31.98	32.34	32.42	32.22	P<0.05	1.35
Specific gravity (g/cm^3)	1,077	1,074	1,075	1,074	1,075	1,075	NS	0.55

CV = Coefficient of variation; NS = Not significant, P>0.05.

tissue may be affected more by changes in blood and liver amino acid concentration (Novak et al., 2004).

Linear effect was observed for shell weight according to equation $\hat{Y} = 0.880402 + 0.0966857x$; $R^2 = 0.74$ and for percentage of shell according to the equation $\hat{Y} = 9.47312 - 1.57543x$; $R^2 = 0.82$. Belo et al. (2000) found a linear decrease in the levels of sulfur amino acids on the percentage of shell and Pinto et al. (2003) found a quadratic effect relationships of methionine plus cystine with lysine on this parameter. According to Fraser et al. (1998), the base of the eggshell consists of a protein matrix. The reduction in the percentage of eggshell can be explained by the fact that the increasing egg weight did not occur as the eggshell deposition increased.

A linear effect was observed for the relationships of methionine plus cystine with lysine on egg diameter according to the equation $\hat{Y} = 25.9623 + 1.67657x$; $R^2 = 0.65$ whereas for the height of the eggs, the increase in sulfur amino acid ratios responded quadratically according to the equation $\hat{Y} = 12.5176 + 47.1024x - 27.9643x^2$; $R^2 = 0.96$, reaching a peak at the ratio of 0.84. By studying the phenotypic correlation among the external characteristics of the egg, Kul & Seker (2004) found a diameter of 25 mm and a height of 33.4 mm, and reported that the egg shape has a positive correlation with the diameter of 0.34 and a negative one with the height of 0.77. This indicates that the quail egg tends to be more rounded than elongated.

Relationships of methionine plus cystine with lysine did not influence the result of specific gravity. This indicates that, although there was an increase in egg weight and decrease in percentage shell, it did not result in change on the quality of the eggshell.

Conclusions

The relationship of methionine plus cystine with lysine of 0.84 which corresponds to the consumption of 221.0 mg of methionine + cystine/bird/day provides better performance and satisfactory quality of Japanese quail egg.

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