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# *Lithothamnium calcareum* in the diet of Japanese quails improves the external quality of eggs

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## Abstract

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This study aimed to evaluate the use of seaweed *Lithothamnium calcareum* for Japanese quails to replace the inorganic calcium source commonly used (limestone) and verify its influence on quails' productive performance and egg quality. A total of 168 Japanese quails were distributed in a completely randomized experimental design, with 4 treatments: control feed and diets containing the inclusion of 3 different levels of calcareous seaweed (10%, 20%, and 30%) to replace limestone. Regarding performance, the lowest feed intake by using the equation was 16.24% of inclusion. The inclusion of calcareous seaweed showed a quadratic effect for the parameters egg weight, albumen height, and albumen weight, and from the derivative of the equations, the best performance point for these variables were obtained at 15.61%, 16.06%, 15.65% respectively. Regarding shell thickness and strength, there was an increasing linear effect ($p < 0.05$) for the levels used, the higher level of calcareous seaweed in the diet, the greater thickness and force applied to break the shell. The inclusion of 15% of calcareous seaweed flour in Japanese quails' diet results in heavier eggs and when up to 30% is added there is an increase in shell thickness and strength.

*Keywords:* calcium; coturniculture; rock flour; organic minerals

## Introduction

The interest in quail farming has increased in recent years. The egg is the main product of this type of breeding and is a source of animal protein with high biological value. Egg quality is determined by several characteristics, external and internal, which together guarantee its degree of acceptability by consumers (Feddern et al., 2017). Among these characteristics, shell quality is one of the most important, being defined by its integrity, resistance, and deformations (Samiullah et al., 2017). Several factors are responsible for problems with eggshells such as room temperature, genetics, age, and especially nutrition (Figueiredo Júnior et al., 2013).

Minerals are one of the most important nutrients, being essential for animal development. Calcium is the major mineral that works in laying bird's performance, due to its requirement to make up eggshells. The shell is composed of 98.2% calcium carbonate; 0.9% magnesium carbonate; and 0.9% calcium phosphate (Silva et al., 2017). The minerals used in animal feed can be obtained in the inorganic source (rocks) or organic sources (bone meal, shells, and algae), in which the inorganic ones such as limestone and dicalcium phosphate are the most used in the feed because they have lower cost and greater availability (Melo & Moura, 2009).

However, inorganic sources of calcium are non-renewable mineral resources and their extraction promotes a huge environmental impact. Organic sources can supply the calcium requirements in birds' diets due to the higher effective bioavailability of nutrients (Melo et al., 2008). Calcium obtained from this source has easy absorption, without showing ionic antagonism (Algarea Mineração, 1997). Seaweed flour is obtained from the skeleton of the seaweed *Lithothamnium calcareum*, which is a rich source of calcium carbonate and contains in its structure magnesium and more than 20 trace elements such as iron, manganese, boron, nickel, copper, zinc, molybdenum, selenium and strontium (Dias, 2000).

The limestone produced by the extraction of these algae is known as biogenic limestone and can be used in soil correction and fertilization, animal and human diets, and the cosmetic industry (Carlos et al., 2011). The great insertion of agro-sustainable, agroecological, and organic systems is present in industrial poultry, more and more consumers are concerned with how food is produced. In poultry farming due to intensive production, where birds are produced in confined cages, this aspect is even more visible. Thus, agroecological production is expanding rapidly (Jaenisch et al., 2010).

Organic production has some peculiarities to other breeding systems, according to Azevedo et al. (2016) in addition to having organic certification, the use of pesticides, synthetic chemical fertilizers is not allowed, the entire breeding system must be at the same time open air, that is, confinement is reprehensible, food must come from organic production, without the use of transgenic grains and the animals acquired must be resistant to pathogens.

Calcarea seaweed is a product based on *Lithothamnium*, which is extracted from sedimented seaweed. These algae are renewable sources and are found on the coast of Brazil. It is important to note that this product is extracted from sedimented algae and not live algae that are part of the marine ecosystem.

In the nutritional aspect, the use of organic minerals goes hand in hand with this production, due to the lesser environmental impact caused by the industrial production of inorganic mineral substances resulting from mineral extraction.

The use of seaweed flour (*Lithothamnium calcareum*) is an alternative with less environmental impact, as it is a renewable mineral source (Carlos et al., 2011). The objective of this study was to evaluate the use of the seaweed *Lithothamnium calcareum* (organic source) for Japanese quails to replace the inorganic calcium source commonly used (limestone) and verify its influence on quails' productive performance and egg quality.

## Material and Methods

The experiment was carried out in the Quail Department of the Agricultural Sciences Faculty Research Unit at Federal University of Grande Dourados (FCA / UFGD), Dourados – MS, for 84 days, divided into three experimental periods of 28 days each. All procedures and management of the animals carried out under the consent and approval of the Ethics Committee on the Use of Animals of the University Center of Grande Dourados (Unigran/Dourados), under protocol number 052/18. 168 Japanese quails (*Coturnix japonica*), 200-day-old, laying rate of 85%, with an average weight of 138 g were used. The quails received the experimental diet 7 days before starting the experimental period.

Quails were housed in galvanized wire cages measuring 35 x 25 x 20 cm (length x width x height) providing an area of 175 cm²/Bird located in a conventional housing system, with cages in parallel, on five floors with two air conditioners, to control temperature and humidity. Feeders were gutter type and for water supply, nipple drinkers were used.

Quails were distributed in a completely randomized design, with four treatments, and seven replicates of six birds per experimental unit. Calcareous seaweed was purchased from a commercial company. Temperature (TºC) and relative humidity (RH) were observed twice a day, at 8:00 am and at 4:00 pm using a digital thermohygrometer placed in the center of the barn.

In the morning, the minimum temperature obtained was 19.4 +- 0.3 (°C), and maximum temperature of 27.6 + -0.2 (°C), a maximum humidity of 76.6 + -1.3 (%), and a minimum humidity of 49.1 +- 1.4 (%). In the afternoon, the temperature average registered was 22.6 +- 0.2 (°C), maximum temperature of 32.4 + – 0.3 (°C), maximum humidity of 78.9 +- 1.4 (%) and minimum relative humidity of 33.7 +- 1.5 (%). 16 hours of daily light (natural + artificial) were provided throughout the experimental period and controlled by an automatic clock (timer).

According to the temperature and relative humidity values obtained, quails went through a period of heat stress, because the conditions of thermal comfort are close to 21°C and the relative humidity between 57 to 69% (Dos Santos et al., 2017). However, the birds had no losses in productive performance, showing to be adapted to the breeding environment.

Experimental diets (Table 1) were formulated according to nutritional requirements established by Rostagno et al. (2017). The treatments were: 0 – Control (basal ration, without calcareous seaweed flour); 10 – Basal ration + 10% calcareous seaweed flour replacing limestone; 20 – Basal feed + 20% calcareous seaweed flour replacing limestone and 30 – Basal feed + 30% calcareous seaweed flour replacing limestone.

The water was supplied *ad libitum*. Feed was provided twice a day, 40 g of feed per quail/day. Table 2 demonstrates the chemical composition of calcareous seaweed and limestone, reported by Dias et al. (2000).

Eggs were daily collected at 8:00 am. Egg production percentage was obtained by the number of eggs produced, including broken, cracked, eggs with soft shells and shell-less produced in each experimental period being expressed as a percentage on the average number of birds in the period (egg/quail/day).

All eggs produced by each experimental unit were weighed in the last three days of each experimental period. Mortality was recorded and the number of deaths was subtracted from the total number of live birds, with the values converted into a percentage at the end of the experimental period.

**Table 2. Chemical composition of calcareous seaweed and limestone**

| Chemical composition | Calcareous seaweed | Limestone |
|---|---|---|
| Ashes, % | 95.00 | 97.70 |
| Calcium, % | 32.39 | 39.90 |
| Magnesium, % | 5.00 | 0.32 |
| Sulfur, % | 0.25 | ----- |
| Sodium, % | 0.13 | ----- |
| Potassium, % | 0.01 | ----- |
| Chlorine, % | 0.10 | ----- |
| Phosphorus, % | 0.02 | ----- |
| Boron, ppm | 10.00 | ----- |
| Iron, ppm | 125.00 | 90.00 |
| Copper, ppm | 725.00 | ----- |
| Zinc, ppm | 5.50 | ----- |
| Manganese, ppm | 10.00 | ----- |
| Molybdenum, ppm | 2.50 | ----- |
| Selenium, ppm | 0.50 | ----- |
| Iodine, ppm) | 6.00 | ----- |

*Adapted*: Dias et al. (2000).

**Table 1. Calculated composition of experimental diets**

| Ingredients, % | Replacement of calcareous seaweed flour, % | | | |
|---|---|---|---|---|
| | 0.00 | 10.00 | 20.00 | 30.00 |
| Corn | 54.17 | 54.17 | 54.17 | 54.17 |
| Soybean meal | 34.70 | 34.70 | 34.70 | 34.70 |
| Limestone | 7.01 | 6.30 | 5.60 | 4.90 |
| Dicalcium phosphate | 1.15 | 1.15 | 1.15 | 1.15 |
| Sodium chloride | 0.36 | 0.36 | 0.36 | 0.36 |
| Premix[1] | 1.50 | 1.50 | 1.50 | 1.50 |
| Soybean oil | 1.11 | 1.11 | 1.11 | 1.11 |
| Calcareous seaweed flour | 0.00 | 0.70 | 1.40 | 2.10 |
| Total | 100 | 100 | 100 | 100 |
| Nutritional composition calculated | | | | |
| Metabolizable energy (kcal/kg) | 2800.00 | 2800.00 | 2800.00 | 2800.00 |
| Crude protein (%) | 19.46 | 19.46 | 19.46 | 19.46 |
| Digestible Lysine (%) | 1.08 | 1.08 | 1.08 | 1.08 |
| Digestible Met+Cist (%) | 0.94 | 0.94 | 0.94 | 0.94 |
| Digestible Tryptophan (%) | 0.23 | 0.23 | 0.23 | 0.23 |
| Digestible Threonine (%) | 0.68 | 0.68 | 0.68 | 0.68 |
| Available Calcium (%) | 3.07 | 3.07 | 3.07 | 3.07 |
| Available Phosphorus (%) | 0.30 | 0.30 | 0.30 | 0.30 |
| Sodium (%) | 0.16 | 0.16 | 0.16 | 0.16 |
| Crude fiber (%) | 2.74 | 2.74 | 2.74 | 2.74 |

Guarantee levels per kg of Premix: Folic Acid (min.) 900.0 mg; Pantothenic acid (min.) 12,000.00 mg; Biotin (min.) 77.0 mg; Calcium (min – max) 130.0 – 143.7g; Niacin (min.) 40,000.0 mg; Selenium (min.) 370.0 mg; Vitamin A (min.) 8,800,000.0 IU; Vitamin B1 (min.) 2,500.0 mg; Vitamin growth 0.04 g; Antioxidant 0.02 g; Mn 75 mg; Zn 50 mg; Cu 8 mg; I 0.75 mg; Fe 50 mg. Copper (min.) 7,000.0 mg; Iron (min.) 50.0 g; Iodine (min.) 1,500.0 mg; Manganese (min.) 67.5 g; Zinc (min.) 45.6 g.

Feed intake was determined by the amount of feed consumed considering the number of quails for each treatment. The feed conversion per dozen eggs was calculated by the ratio of the total feed intake in kg divided by the dozen eggs produced (kg/DZ) and the feed conversion per egg mass calculated by the feed intake in kilograms divided by the total egg mass (kg/kg).

Three eggs were collected during three days from the end of each of the three experimental cycles, in the morning, totaling 756 eggs analyzed. Eggs were analyzed at the Meat Technology Laboratory of FCA/UFGD and were individually weighed on a digital scale with an accuracy of 0.01g. Specific gravity was determined by immersing the eggs in saline solutions, with density ranging from 1.065 to 1.100 g $cm^{-3}$, with intervals of 0.005. The eggs were submerged in each saline solution, in increasing order of gravity, beginning in the lower density solution, according to the methodology described by Bittencourt et al. (2019). Specific gravity was represented by the lower density solution in which the egg floated.

Eggs were broken and the shells, yolk, and albumen were separated on a flat surface to proceed with the analysis. First, the color evaluation was performed using the Minolta CR 410 portable colorimeter, where the parameters L * (luminosity), a * (red), and b * (yellow) were evaluated. The readings were measured at three different points on the surface of the egg yolk.

The height of the yolk and albumen and diameter of the yolk were measured using a digital caliper and a tripod. Yolk height was measured in the middle region and the height of the albumen at about 1 cm from the yolk. Egg constituents were weighed on a digital scale with an accuracy of 0.01 g to determine the percentage of shell, egg, and albumen, considering total egg weight. Shells were washed and dried in a forced ventilation oven at 65°C for 72 hours, then they were weighed on a digital precision scale and the percentage of shells about the total weight of the eggs was calculated.

Haugh unit was calculated using a mathematical model, according to Alleoni & Antunes (2001) methodology:

$$UH = 100 \log (H + 7.57 – 1.7W0, 37),$$

where: H = height of dense albumen (mm); W = egg weight (g).

The yolk index was determined by the ratio between the height and the diameter of the yolk. The pH of the yolk and albumen was obtained using a digital pH meter.

To evaluate breaking strength (KJ/F), 3 samples were randomly collected from each experimental unit, totaling 252 eggs evaluated. The force required to break the shell (g) was determined using the Food Texturometer – TAX T2 Texture Analyzer (Stable Micro Systems, Surrey, England). A stainless steel P4 DIA Cylinder probe, 4 mm in diameter was used, with pre, during and post-test speed of 3.0; 0.5; and 5.0; mm/s and a distance of 6 mm with a trigger force of 3.0 g. A compression fracture test was used, in which the whole egg is placed longitudinally (Rodriguez-Navarro, 2002) on ring-shaped metal support (5 cm in diameter) inside a porcelain crucible and the shell is pressed until the fracture occurs.

The parameters analyzed were submitted to the statistical premises of normality of residues through the Shapiro Wilk test and the homogeneity of variances through the Levene's test. Data showing disparity changed and then the analysis of variance was performed using the R Studio.Ink ® program. Regression analysis was performed using linear and quadratic models for the data found at the 5% probability, the contrasts of orthogonal polynomials were used and the regression equations were adjusted.

## Results

There was a positive quadratic effect for the parameter feed intake ($Y = 29.277 -0.203x + 0.00629x^2$. $R^2$: 98.32%), which means that with the level of replacement of 16.24%, the lowest feed intake is observed. There were no significant differences between treatments for the parameters feed conversion (Mass and Dozen), egg production, and viability (Table 3).

**Table 3. Performance of Japanese quails fed diets with different levels of calcareous seaweed**

| Parameters | Replacement of calcareous seaweed flour, % | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | CV% | SEM | p-value |
| Feed intake, g/quail/day² | 29.31 | 27.75 | 27.83 | 28.78 | 4.55 | 1.92 | 0.0116 |
| Feed conversion per mass | 2.79 | 2.54 | 2.657 | 2.62 | 9.12 | 0.43 | 0.0861 |
| Feed conversion per dozen | 2.99 | 2.84 | 2.781 | 2.80 | 10.94 | 0.36 | 0.3337 |
| Egg production, % | 88.96 | 91.35 | 92.49 | 93.24 | 8.46 | 3.21 | 0.5564 |
| Marketable eggs, % | 87.76 | 90.34 | 91.45 | 92.87 | 5.69 | 3.46 | 0.4580 |
| Viability, %* | 98.00 | 98.60 | 99.60 | 99.60 | --- | --- | --- |

²quadratic effect ($P < 0.05$); CV: Coefficient of variation. SEM: standard error of the mean. * descriptive analysis. Feed intake: $Y = 29.277 -0.203x + 0.00629x^2$. $R^2$: 98.32% (16.24% of inclusion)

With the level of replacement of 16.24%, the lowest feed intake was observed by the equation. This fact can be explained because of the greater bioavailability of algae, which attend quail requirements without compromising other performance factors, such as feed conversion.

A positive quadratic effect ($p < 0.05$) was observed between treatments for the average egg weight ($Y = 11.934 + 0.0217x + 0.000695x^2$; $R^2$: 94.91%), albumen height ($Y = 4.336 + 0.0530x - 0.00165x^2$; $R^2$: 94.57%), albumen weight ($Y = 7.529 + 0.0212x + 0.000679x^2$; $R^2$: 91.21%) and increasing linear effect ($p < 0.05$) for shell thickness ($Y = 0.2936 + 0.4588x$; $R^2$: 68.02%) and for shell strength ($Y = 0.7756 + 0.0632x$; $R^2$: 61.74%). The other parameters did not show an effect between treatments (Table 4).

The inclusion of calcareous seaweed flour showed a quadratic effect for the parameters egg weight, albumen height, and albumen weight, and from the derivative of the equations, the best performance point for these variables were obtained at 15.61%, 16.06%, 15.65%, respectively.

## Discussion

Egg production results were between 88.96% and 93.24% without difference among treatments, corroborating with results obtained by Melo et al. (2008) who evaluated the use of 0.25% and 0.50% of seaweed *Lithothamnium calcareum* flour in the performance of Japanese quails at 26 weeks of age and did not found influence on egg production.

However, these results contrast with the results found by Perali et al. (2003), who observed an increase in the production of Japanese quail eggs by 4.16% when using only 0.25% of *Lithothamnium calcareum* comparing with control treatment. It can be explained by the calcium bioavailability of the calcareous algae being well absorbed by the quails' organism (Sousa et al., 2017), avoiding mineral deficiencies and making it possible to obtain similar performance similar when using common calcium sources in the diet.

For the parameter marketable eggs (eggs without cracks, broken, or shelled) there was no influence from the replacement of calcareous seaweed, which shows the ability of this calcium source to supply the quails' calcium requirement. Londero (2019), comparing the effect of organic microminerals and calcareous algae on performance, egg quality, incubation, and sperm quality of poultry breeders, concluded that supplementation with calcareous algae improves eggshell quality and does not affect the performance of the birds, corroborating with this research.

**Table 4. Egg quality of Japanese quails fed diets with different calcareous seaweed levels**

| Parameters | Replacement of calcareous seaweed flour, % | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | CV% | SEM | p-value |
| Average egg weight, g[2] | 11.84 | 12.05 | 11.39 | 11.71 | 7.10 | 0.59 | 0.009 |
| Haugh unit | 87.76 | 91.17 | 89.52 | 89.04 | 6.17 | 1.54 | 0.074 |
| Albumen height, mm[2] | 4.26 | 4.90 | 4.52 | 4.50 | 20.98 | 0.23 | 0.043 |
| Albumen weight, g[2] | 7.44 | 7.57 | 6.99 | 7.36 | 11.11 | 0.11 | 0.021 |
| Yolk weight, g | 3.61 | 3.67 | 3.61 | 3.58 | 12.76 | 0.09 | 0.853 |
| Shell weight, g | 0.79 | 0.80 | 0.89 | 0.89 | 12.43 | 0.06 | 0.933 |
| % Albumen | 62.76 | 62.77 | 61.36 | 62.77 | 6.74 | 0.72 | 0.392 |
| % Yolk | 30.54 | 30.54 | 31.80 | 30.66 | 12.40 | 0.12 | 0.433 |
| % Shell | 6.66 | 6.71 | 6.96 | 6.79 | 14.14 | 0.03 | 0.559 |
| Yolk diameter | 24.50 | 24.70 | 24.65 | 25.34 | 5.80 | 0.29 | 0.072 |
| Yolk index | 0.280 | 0.27 | 0.27 | 0.28 | 9.78 | 0.01 | 0.139 |
| L | 55.60 | 54.45 | 54.14 | 54.25 | 5.05 | 1.95 | 0.097 |
| a* | -1.90 | -1.58 | -1.85 | -2.10 | 5.90 | 0.03 | 0.083 |
| b* | 42.02 | 39.39 | 40.80 | 40.09 | 11.75 | 1.45 | 0.118 |
| Shell thickness, mm[1] | 0.43 | 0.44 | 0.50 | 0.55 | 13.65 | 0.06 | 0.032 |
| Strength test, KJ/F[1] | 0.83 | 0.90 | 0.96 | 1.02 | 21.44 | 0.14 | 0.0041 |
| Yolk pH | 6.38 | 6.35 | 6.33 | 6.51 | 5.17 | 0.88 | 0.096 |
| Albumen pH | 8.912 | 8.942 | 8.948 | 9.076 | 3.09 | 0.03 | 0.069 |
| Specific gravity, g/cm[3] | 1.06 | 1.07 | 1.06 | 1.07 | 0.48 | 0.04 | 0.9882 |

[1]Linear effect ($P < 0.05$); [2]Quadratic effect ($P < 0.05$); CV: Coefficient of variation. SEM: standard error of the mean. Regression equation: Average egg weight: $Y = 11{,}934 + 0.0217x + 0.000695x^2$; $R^2$: 94.91% (15.61% of inclusion). Albumen height: $Y = 4{,}336 + 0.0530x - 0.00165x^2$; $R^2$: 94.57% (16.06% of inclusion). Albumen weight: $Y = 7.529 + 0.0212x + 0.000679x^2$; $R^2$: 91.21% (15.65%). Shell thickness: $Y = 0.2936 + 0.4588x$; $R^2$: 68.02%. Shell strength = $0.7756 + 0.0632x$; $R^2$: 61.74%.

Carvalho et al. (2016) studying the inclusion of different levels of seaweed flour (*Lithothamnium calcareum*) in commercial laying hens' diet, found good results when using 1% of seaweed flour, obtaining better egg production percentage and shell thickness improvement, with a higher percentage of mineral matter and calcium in the shell, and a lower percentage of cracked eggs.

When minerals reach the gastrointestinal tract, they must first be solubilized to release ions and be absorbed (Costa Neto et al., 2010). However, being in ionic form, they can complex with other components of the diet, making absorption more difficult or unavailable to animals (Moraes et al., 2005). According to Fassani et al. (2004), the lack of knowledge of the physical-chemical characteristics of mineral sources can cause differences in the nutritional requirements obtained in scientific research, which leads many nutritionists to use higher calcium levels on diets. The use of eggshell flour replacing partial or total limestone in the quail diet showed a positive effect on egg weight, egg mass, and feed conversion rates per egg mass (Ribeiro et al., 2015).

The interest in organic minerals has increased in recent years, especially because minerals are absorbed by intestinal carriers of amino acids and peptides and not by intestinal mineral carriers (Albuquerque et al., 2019). This fact avoids minerals competition for the same absorption sites and consequently increases the availability of minerals (Rutz et al., 2007). These studies make the benefits of using organic minerals in the animal body more clearly. In the present research, the inclusion of up to 30% of calcareous algae in Japanese quail's diets can replace limestone without negative impacts on performance.

The results obtained for average egg weight and albumen weight differ from the results obtained by Perali et al. (2003), who did not observe an increase in the weight of quail eggs receiving diets with seaweed flour. This result is probably because the seaweed flour does not influence the weight of the albumen, which is responsible for approximately 60% of the weight of the whole egg. It is assumed that greater deposition of albumen and shell is due to the bioavailability of minerals, obtaining a higher weight of eggs. Stands out among the microminerals: iron, zinc, manganese, and copper, which are responsible for some physiological processes, and works as essential cofactors for many cellular enzymes directly associated with growth and development of bone tissue and bird's eggshell (Richards et al., 2010).

There was an increasing linear effect ($p < 0.05$) by the levels used for the parameters shell thickness ($Y = -0.2936 + 0.4588x$) and shell strength ($Y = 0.7756 + 0.0632x$; $R^2$: 61.74). Higher levels of calcareous algae in the diet promote greater thickness and higher force needs to be applied to break it. Shell thickness is defined by the layers that make up its structure and has a direct influence on the internal preservation of eggs. The greater shell thickness promotes resistance of the egg for storage and when handling, transporting (Saccomani et al., 2019).

Shell quality has an impact on egg quality, and it must be strong enough to prevent cracks and breaks (Bittencourt et al., 2019). Changes in thickness increase the probability of losses in the integrity of the shell. Eggs cracked in different degrees, eggs cracked in the uterus, eggs with thin or absent shells are examples of integrity imperfections (Chukwuka et al., 2011). Cracked and broken eggs result in great economic damage to producers. Such losses are difficult to measure due to the lack of standards and control during the production process (Freitas et al., 2020).

The greater bioavailability of calcium in the algae structure contributes to an increase in shell thickness because the connection with organic molecules makes absorption easier (Murata et al., 2009). Regarding the parameters Haugh unit, albumen and yolk pH, yolk percentage, shell percentage, albumen percentage, yolk diameter, yolk index, L, a *, b * and specific gravity, there was no significant effect ($p > 0.05$) between the treatments analyzed. The results found corroborates with Figueiredo Júnior et al. (2009) study, which did not find influences between organic and inorganic minerals in yolk percentage and Haugh unit.

According to Silva et al. (2017) the use of seaweed significantly reduced ($P < 0.05$) the average daily feed intake, increased ($P < 0.05$) the weight and mass of eggs and improved ($P < 0.05$) feed conversion per dozen and egg mass, which influenced Haugh unit results. The aforementioned authors pointed out that the use of calcareous seaweed flour in laying hens at the beginning of production showed an improvement in product performance and the internal quality of eggs.

According to Alvarenga et al. (2011), *Lithothamnium* can be used to replace other sources of calcium. The calcium required to build the shell just comes from the diet, being carried through the bloodstream in the ionic form of calcium or linked to a phosphoprotein (Arruda et al., 2015). Calcium is extremely important to build the eggshell. One egg has about three grams of calcium, which confirms the necessity of providing an adequate supplementation of this mineral for laying birds. As observed in the present study, the organic source of calcium showed better absorption by quails compared to the inorganic source (Cedro et al., 2011).

*Lithothamnium calcareum* composition has between 32% and 38% calcium and 2% magnesium (Carlos et al., 2011). The seaweed structure is very porous, with 60% empty spaces, which provides high solubility (Junior et al., 2018). The high bioavailability and solubility of the calcareous seaweed components were responsible for better egg quality, especially because of the higher egg and shell weight.

The great growth of organic agriculture in the country is also one of the justifications for the use of organic minerals. As reported by Souza Azevedo et al. (2016) the consumer market is increasingly concerned with the foods of animal origin that they consume and especially in the conditions in which they are kept during the production period. Proposals for changes in the current poultry production systems are being implemented to meet the market requirements regarding the ways of raising, giving satisfactory profits to producers who adopt the systems of free-range and organic farms.

Organic production has some particularities about other conventional and free-range farming systems, in addition to having the organic certification, the use of pesticides and synthetic chemical fertilizers is not allowed and aims to increase biodiversity and biological cycles, better reaching the natural systems aiming at sustainability (Souza Azevedo et al., 2016).

Products considered organic are produced in an environment based on the production process, the agroecological principles that include the responsible use of soil, water, air, and other natural resources, respecting social and cultural relations (MAPA, 2015). According to the Ministry of Agriculture, in organic agriculture, the use of substances that endanger human health and the environment is not allowed. Thus, organic minerals meet these requirements and can be indicated for use in organic poultry.

In Brazil, a country with great agricultural potential and a long tradition in family farming, such development is slow because although it is in fifth place in the area (ha) destined for organic production in the world, there are no organic products of animal origin, such as meat, milk, and eggs marketing quality, in sufficient quantities, at affordable prices for the Brazilian population (Figueiredo & Soares, 2012). In this regard, the creation of quails is an alternative activity with a great market perspective for organic production, aiming to serve a growing market niche in the world, given the large consumption of eggs on a global scale. For small producers, cotton farming is an area of great growth. These birds have characteristics of early growth and high productivity, being of great relevance for organic production, as a secondary activity.

## Conclusions

The inclusion of 15% of calcareous seaweed flour in Japanese quail diets results in heavier eggs and when up to 30% is added, there is an increase in shell thickness and strength. The replacement of up to 30% of calcareous seaweed in the Japanese quail diet can be recommended because improves the external quality of the egg, being an alternative to reduce economic losses from egg breaks and cracks.

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