

# Performance and egg quality of Japanese quail fed diets containing microalgae *Schizochytrium* sp.

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**ABSTRACT** - A trial was carried out to evaluate the effects of different levels of microalgae *Schizochytrium* sp. on performance, yolk lipid profile, and egg quality of Japanese quail. A flock of 210 quail was distributed in a completely randomized design, with five treatments (0, 10, 20, 30, and 40 g of *Schizochytrium* sp./kg of feed) and six replications with seven birds per cage. Performance and egg quality were not affected, except for a quadratic effect on yolk color, which reached the maximum value with the inclusion of 40 g of *Schizochytrium* sp./kg. There was linear reduction in the content of saturated fatty acids and a linear increase of polyunsaturated:saturated and polyunsaturated:monounsaturated ratios and n-6. The content of n-3 showed a minimum value with the inclusion of 6.5 g of *Schizochytrium* sp./kg, and the n-6:n-3 ratio was maximized with the addition of 10.5 g of microalgae/kg. As for the sensory attributes color, aroma, and overall impression, there was linear increase with the addition of increasing levels of microalgae. The inclusion of up to 40 g of microalgae *Schizochytrium* sp./kg in the diet of Japanese quail did not present changes in the performance nor in the egg quality but accentuated the yolk color, promoted the fortification of n-3 in the eggs, and still provided excellent sensorial acceptance. The egg fortification can add value to the product, increasing the producer remuneration and improving the nutritional quality of the diet for humans.

**Keywords:** animal nutrition, fatty acids, fat deposition, feed quality, omega 3

## 1. Introduction

In recent years, the poultry and egg industries have explored nutritional strategies in the formulation of poultry diets, by modifying the composition of the yolk lipids (Brelaz et al., 2019). Therefore, the eggs can be considered a reference model for transferring nutrients from the diet, once the yolk fatty acid type is related to the consumed in the feed.

In countries that need to improve sustainable rural development, the search for alternative, environmentally adequate, and efficient food resources is highly relevant. Hence, the inclusion of algae in animal feed forms a foundation, due to its specificities and its importance in sustainable rural development, as it is a renewable source.

The use of algae in animal nutrition has become a potential possibility, because they directly produce polyunsaturated fatty acids (PUFA) such as those present in the n-6 and n-3 series, important lipids present in fish oil and meal (Shields and Lupatsch, 2012).

Kralik et al. (2020) demonstrated that the nutrient content of eggs can be altered by a simple technological process, adding certain ingredients into the laying poultry feed. The fatty acid profile in diets influences the fatty acid profile in egg yolks.

According to Fernandes et al. (2020), although microalgae have desirable levels of bioactive compounds that can be used in the nutrition of laying hens, the patterns of deposition and egg enrichment may not be proportional to the inclusion level or period of dietary supplementation. So, it is necessary to develop research that can fill these gaps.

Thus, the hypothesis of this study is that the efficiency of egg enrichment with n-3 PUFA will be higher when using greater amount of microalgae in the quail diet, without harming animal performance or sensory attributes.

Quail egg production is a segment that is booming. When considering the development of this activity, the increase in the number of quail housed and in annual egg consumption per capita stand out.

Since most of the studies related to egg fortification with PUFA n-3 were conducted with hens, together with the lack of published results for quail, the need of further research for this species is evident. In this sense, the objective of this study was to evaluate the effects of different levels of microalgae *Schizochytrium* sp. on performance, yolk lipid profile, and egg quality of Japanese quail.

## 2. Material and Methods

The research was conducted in Rio Pomba, Minas Gerais, Brazil (21°16'45" S, 43°10'30" W, 434 m) during 42 days divided into three periods of 14 days, after being approved by the Ethics Committee on Animal Use (case number 09/2016).

The nutritional composition of the microalgae *Schizochytrium* sp. was analyzed by the company that supplied the product (Table 1). The diets were formulated based on corn and soybean meal (Table 2), isonutritive, with the requirements and nutritional composition based on the recommendations of Rostagno et al. (2017). The nutritional profile of the microalgae was considered in the balance of the experimental diets.

Birds were housed in galvanized wire cages (25 L × 16.5 H × 50 W, cm), with an animal density of 178 cm<sup>2</sup>/quail, equipped with gutter-type feeders and nipple drinkers, and had free access to feed and water.

The daily practice consisted of collecting and counting eggs, providing ration three times a day, cleaning egg trimmers, reading temperatures (maximum and minimum) and relative humidity (RH), and providing 16 h of light daily, during the entire trial period.

The evaluated productive parameters were feed intake (g/d), feed conversion (kg/kg eggs and kg/dozen eggs), egg mass (g/bird/d), egg production (%), marketable egg production (%; whole eggs/total eggs produced/number of live quail × 100), and bird viability (%). The number and weights of eggs were recorded daily during the study period, and egg mass was calculated by multiplying the number of laid eggs by their weight.

Normal eggs were randomly selected from the eggs laid during the final three days of each period of 14 days (five groups × six replicates per group × four eggs per replicate) to assess the following qualitative traits: weight (g), specific gravity (g/cm<sup>3</sup>), yolk and albumen weight (g) and percentage (%), eggshell weight (g) and percentage (%), and yolk color. The specific gravity of eggs was determined by immersing them in saline solutions of different densities (1.05-1.09 g/cm<sup>3</sup>). Albumen weight (g) was calculated as the difference between the weight of the entire egg and the combined weight of the yolk and eggshell (g). Percentages of yolk, albumen, and eggshell were determined by the following formula:

yolk (%) = [yolk weight (g)/egg weight (g)] × 100. Albumen and eggshell weights were substituted in the formula, as necessary. The evaluation of yolk color was performed subjectively with the use of the DSM® colorimetric fan (score from 1 to 15, which varies from light yellow to orange).

Egg fatty acid profile was determined at the end of the experimental period, on the forty-third day. The determination of fatty acid profile in the yolks (twenty-four yolks per group) was carried out by gas chromatography, using the direct methylation method, according to Wang et al. (2000).

To perform the sensory test, the project was previously submitted to the Human Research Ethics Committee and was approved with CAAE registration no. 62071716.7.0000.5588. Fifty-three evaluators of both sexes, over 18 years of age, untrained, and voluntarily recruited, participated in the test. They were aware of the voluntary participation through a Free and Informed Consent Term, which contained data on the research, risks, and benefits of participation.

On the day of the sensory analysis, quail eggs were boiled and served cut longitudinally, containing albumen and yolk, as described by Moraes (1985). The tasters were accommodated in individual, clean cabins, free from noise and odors with good ventilation and lighting, as recommended by ABNT (2014).

The acceptability test was applied, and the affective quantitative method (Meilgaard et al., 1999) was used, to evaluate the attributes, color, aroma, flavor, and global acceptance of eggs, through a nine-point mixed structured hedonic scale (starting from 1 - “I really disliked it” to 9 - “I liked it a lot”), and intention to buy the product with a scale ranging from 1 - “decidedly not buying” to 5 - “decidedly buying”.

**Table 1** - Nutritional composition of the microalgae *Schizochytrium* sp., as dry matter<sup>1</sup>

| Nutritional composition          | g/kg  |
|----------------------------------|-------|
| Dry matter                       | 963.0 |
| Crude protein                    | 192.2 |
| Ether extract                    | 500.0 |
| Crude fiber                      | 9.0   |
| Ash                              | 36.7  |
| Total phosphorus                 | 4.7   |
| Total calcium                    | 3.4   |
| Fatty acid                       | g/kg  |
| Saturated                        |       |
| Myristic (C14:0)                 | 38.6  |
| Palmitic (C16:0)                 | 546.9 |
| Margaric (C17:0)                 | 6.3   |
| Stearic (C18:0)                  | 18.0  |
| Arachidic (C20:0)                | 2.8   |
| Monounsaturated                  |       |
| Myristoleic (C14:1n9)            | 16.0  |
| Polyunsaturated                  |       |
| Eicosapentaenoic (EPA; C20:5 n3) | 2.8   |
| Erucic (C22:1 n9)                | 5.3   |
| Docosadienoic (C22:2 n6)         | 4.3   |
| Docosahexaenoic (DHA; C22:6 n3)  | 272.0 |
| Other fatty acids <sup>2</sup>   | -     |
| Not identified                   | 7.1   |
| EPA + DHA                        | 274.8 |

<sup>1</sup> All-G-Rich (Alltech Inc.®).

<sup>2</sup> These fatty acids were detected in concentrations below 0.005%: caproic (C6:0), heptanoic (C7:0), caprylic (C8:0), nonanoic (C9:0), capric (C10:0), undecanoic (C11:0), lauric (C12:0), tridecanoic (C13:0), pentadecanoic (C15:0), palmitoleic (C16:0), margaroleic (C17:1), vaccenic (C18:1 n7), oleic (C18:1 n9), elaidic (C18:1 n9t), linoleic (C18:2 n6), linolelaidic (C18:2 n6t), α-linoleic (C18:3 n3), γ-linoleic (C18:3 n6), nonadecanoic (C19:0), eicosenoic (C20:1 n9), eicosadienoic (C20:2 n6), eicosatrienoic (C20:3 n6), homo-γ-linoleic (C20:3 n6), arachidonic (C20:4 n6), heneicosanoic (C21:0), behenic (C22:0), docosapentaenoic (C22:5 n3), tricosanoic (C23:0), lignoceric (C24:0), and nervonic (C24:1 n9).

**Table 2** - Calculated nutritional composition of the experimental diets (as-fed basis)

| Item  | Levels of <i>Schizochytrium</i> sp. meal (g/kg) |        |        |        |        |
|---|---|--------|--------|--------|--------|
|   | 0   | 10     | 20     | 30     | 40     |
| <b>Ingredient (g/kg)</b>                      |   |        |        |        |        |
| Corn grain                                    | 435.29  | 431.66 | 427.92 | 424.18 | 420.45 |
| Soybean meal (45%)                            | 324.45  | 319.58 | 314.64 | 309.69 | 305.75 |
| Wheat bran                                    | 92.37   | 95.74  | 99.26  | 102.77 | 106.29 |
| Limestone                                     | 60.00   | 60.00  | 60.00  | 60.00  | 60.00  |
| Dicalcium phosphate                           | 40.00   | 40.00  | 40.00  | 40.00  | 40.00  |
| Soya oil                                      | 40.00   | 35.05  | 30.12  | 25.19  | 20.26  |
| Microalgae <i>Schizochytrium</i> <sup>1</sup> | 0.00  | 10.00  | 20.00  | 30.00  | 40.00  |
| Salt  | 3.40  | 3.40   | 3.40   | 3.40   | 3.40   |
| Choline chloride                              | 1.00  | 1.00   | 1.00   | 1.00   | 1.00   |
| L-lysine                                      | 1.90  | 1.95   | 2.00   | 2.06   | 2.11   |
| L-threonine                                   | 0.00  | 0.17   | 0.06   | 0.10   | 0.14   |
| Vitamin-mineral supplement <sup>2</sup>       | 1.50  | 1.50   | 1.50   | 1.50   | 1.50   |
| Antioxidant (BHT)                             | 0.10  | 0.10   | 0.10   | 0.10   | 0.10   |
| Total   | 1000  | 1000   | 1000   | 1000   | 1000   |
| <b>Calculated composition (g/kg)</b>          |   |        |        |        |        |
| Metabolizable energy (kcal/kg)                | 2900  | 2900   | 2900   | 2900   | 2900   |
| Crude protein                                 | 200   | 200    | 200    | 200    | 200    |
| Ether extract                                 | 63.62   | 63.60  | 63.60  | 63.60  | 63.60  |
| Digestible met+cyst                           | 5.48  | 5.43   | 6.19   | 5.32   | 5.27   |
| Digestible lysine                             | 11.12   | 11.05  | 10.97  | 10.90  | 10.82  |
| Total threonine                               | 7.68  | 7.66   | 7.66   | 7.66   | 7.66   |
| Total tryptophan                              | 2.53  | 2.52   | 2.51   | 2.50   | 2.50   |
| Total calcium                                 | 33.46   | 33.49  | 33.51  | 33.54  | 33.56  |
| Available phosphorus                          | 8.78  | 8.76   | 8.76   | 8.76   | 8.76   |
| Sodium  | 1.56  | 1.57   | 1.58   | 1.59   | 1.59   |

BHT - butyl-hydroxy-toluene.

<sup>1</sup> All-G Rich – Alltech Inc.®<sup>2</sup> Vitamin supplement - guaranteed levels per kilogram of product: vitamin A, 8,000 IU; vitamin E, 15,000 mg; vitamin D3, 2300 IU; vitamin K3, 1000 mg; vitamin B1, 200 mg; vitamin B2, 3000 mg; vitamin B6, 1700 mg; vitamin B12, 10,000 mcg; niacin, 20,000 mg; folic acid, 500 mg; biotin, 15.00 mg. Mineral supplement - guaranteed levels per kilogram of product: manganese, 120,000 mg; zinc, 120,000 mg; iron, 60,000 mg; copper, 18,000 mg; iodine, 2000 mg; calcium, 9600 mg.

The statistical model used was:

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

in which  $y_{ij}$  is the response variable measured in the  $j$ -th replicate that received the  $i$ -th treatment,  $\mu$  is the general constant,  $\alpha_i$  is the fixed effect of the  $i$ -th treatment, and  $\varepsilon_{ij}$  is the random error term.

A total of 210 Japanese laying quail (*Coturnix coturnix japonica*, 22th week, and initial body weight of 174.3±12.6 g) were distributed after the uniformity of lots, in a completely randomized design, composed of five treatments (0, 10, 20, 30, and 40 g of *Schizochytrium* microalgae sp./kg of feed), six replicates, and seven birds per experimental unit.

Data obtained for each evaluated parameter was subjected to analysis of variance, and for significant parameters at the 0.05 level of probability, mean comparison was done by Tukey test, and a polynomial regression was performed using the JMP software version 8.01 (SAS Institute, Cary, NC, USA). Linear and quadratic effects are reported where applicable to determine the relationship between the level of microalgae and the analyzed variable.

### 3. Results

Quail breeding was not carried out in an environmentally controlled installation. The average of minimum and maximum temperatures observed inside the installation during the trial were 14.6±1.3

and  $27.3 \pm 3.3$  °C, respectively, and the average relative humidity was  $69.5 \pm 13.7\%$ , being within the standards of thermal comfort for laying quails.

The inclusion of the microalgae *Schizochytrium* sp. did not influence ( $P > 0.05$ ) performance of quail (Table 3). Therefore, the inclusion of up to 40 g of *Schizochytrium* sp./kg did not damage their performance.

The addition of *Schizochytrium* sp. in Japanese quail diet did not affect ( $P > 0.05$ ) egg quality variables, except for yolk color, in which a quadratic effect was observed ( $P < 0.05$ ) (Table 4). This variable reached its maximum value with the inclusion of 40 g of microalgae *Schizochytrium* sp./kg in feed, showing that the PUFA of the microalgae directly influenced yolk pigmentation.

The inclusion of increasing levels of *Schizochytrium* sp. in the diet of Japanese quail promoted the effect ( $P < 0.05$ ) of lipid profile of yolk on saturated fatty acids (SFA), PUFA, PUFA:monounsaturated fatty acids (MUFA) ratio, total omega 6 (n-6), total omega 3 (n-3), and the omega 6:omega 3 ratio (n-6:n-3) (Table 5).

For saturated fatty acids, a linear reduction ( $P < 0.05$ ) in their levels was observed with the microalgae *Schizochytrium* sp. in quail diets. Although there was a linear increase ( $P < 0.05$ ) in PUFA:SFA and PUFA:MUFA ratios. This also occurred for the n-6 content, a linear increase, and for the total n-3 in the egg yolk, the curve was quadratic. When the quadratic equation was derived, the minimum value of n-3 was reached with 6.5 g *Schizochytrium* sp./kg; after that, higher levels of microalgae supplementation promoted greater incorporation of n-3 in eggs (Table 5).

**Table 3** - Mean values of performance of Japanese quail fed diets supplemented with different levels of *Schizochytrium* sp.

| Variable       | Microalgae <i>Schizochytrium</i> sp. (g/kg) |        |       |        |        | P-value | SEM  |
|----------------|---|--------|-------|--------|--------|---------|------|
|                | 0   | 10     | 20    | 30     | 40     |         |      |
| FI (g/bird/d)  | 26.07                                       | 25.57  | 25.97 | 25.97  | 26.46  | 0.54    | 0.35 |
| EP (%/bird/d)  | 87.64                                       | 86.28  | 86.69 | 82.65  | 86.17  | 0.86    | 3.32 |
| MEP (%/bird/d) | 87.19                                       | 85.54  | 86.18 | 81.63  | 85.94  | 0.80    | 3.32 |
| EW (g)         | 10.25                                       | 10.08  | 9.94  | 9.96   | 10.26  | 0.12    | 0.11 |
| EM (g/bird/d)  | 9.00  | 8.69   | 8.62  | 8.19   | 8.85   | 0.56    | 0.35 |
| CM (kg/kg)     | 2.91  | 2.97   | 3.04  | 3.19   | 3.03   | 0.54    | 0.12 |
| CDZ (kg/dozen) | 0.36  | 0.36   | 0.36  | 0.39   | 0.37   | 0.59    | 0.01 |
| VI (%)         | 100.00                                      | 100.00 | 99.20 | 100.00 | 100.00 | 0.43    | 0.35 |

FI - feed intake; EP - egg production; MEP - marketable egg production; EW - egg weight; EM - egg mass; CM - conversion per mass; CDZ - conversion per dozen eggs; VI - viability of quail; SEM - standard error of the mean.

**Table 4** - Mean values for measurements of eggs from Japanese quail fed diets supplemented with different levels of *Schizochytrium* sp.

| Variable                              | Microalgae <i>Schizochytrium</i> sp. (g/kg) |       |       |       |       | P-value | SEM  |
|---------------------------------------|---|-------|-------|-------|-------|---------|------|
|                                       | 0   | 10    | 20    | 30    | 40    |         |      |
| Yolk color <sup>1</sup>               | 4.97d                                       | 5.83c | 6.34b | 6.82a | 6.83a | 0.00    | 0.10 |
| Specific gravity (g/cm <sup>3</sup> ) | 1.074                                       | 1.074 | 1.074 | 1.073 | 1.072 | 0.29    | 0.00 |
| Shell weight (g)                      | 0.82  | 0.81  | 0.79  | 0.78  | 0.81  | 0.34    | 0.11 |
| Shell (%)                             | 8.01  | 7.99  | 8.00  | 7.91  | 7.88  | 0.80    | 0.09 |
| Albumen weight (g)                    | 6.33  | 6.22  | 6.20  | 6.16  | 6.34  | 0.19    | 0.06 |
| Albumen (%)                           | 61.78                                       | 61.67 | 62.37 | 61.88 | 61.78 | 0.48    | 0.28 |
| Yolk weight (g)                       | 3.10  | 3.06  | 2.95  | 3.01  | 3.11  | 0.16    | 0.05 |
| Yolk (%)                              | 30.21                                       | 30.34 | 29.63 | 30.21 | 30.34 | 0.33    | 0.27 |

SEM - standard error of the mean.

<sup>1</sup> Yolk color =  $4.9743 + 0.096x - 0.0012x^2$ ;  $R^2 = 0.99$  ( $P = 0.000$ ).

Means within the same row with different letters differ significantly by Tukey's test ( $P < 0.05$ ).

There was a quadratic effect for the n-6:n-3 ratio in eggs with the inclusion of microalgae *Schizochytrium* sp. in the feed. The addition of the microalgae provided n6:n3 ratio values suitable for human diets, presenting a maximum value with the derivation of the quadratic curve at the inclusion level of 10.5 g of microalgae/kg of feed (Table 5).

Regarding the sensory quality of quail eggs fed diets supplemented with microalgae, no negative results were demonstrated, and in general, the eggs produced by birds that received 40 g of *Schizochytrium* sp./kg of feed were better evaluated (Table 6). The addition of microalgae to quail diet promoted a linear increase in the attributes of color, aroma, and overall impression.

For all the sensory characteristics evaluated in the egg samples, the tasters attributed a score higher than 6 ("I liked it little"), which can be considered the minimum value for the sample, not to be considered as "refused". This demonstrates, therefore, the acceptance of eggs fortified with n-3 from the quail feed, with different levels of the referred microalgae. It is important to note that eggs from quail fed the diet containing 40 g *Schizochytrium* sp./kg had 92.5% marks assigned for global printing within the acceptance zone scores (6 to 9) and no scores in the rejection zone (1 to 4).

The scores for the variable purchase intention (Table 6) were not affected by increasing levels of *Schizochytrium* sp. in the feed. However, it was found that 77% of the tasters (grades 4 and 5) would

**Table 5** - Mean values of content of saturated fatty acids (SFA; %), monounsaturated fatty acids (MUFA; %), polyunsaturated fatty acids (PUFA; %), MUFA:SFA ratio, PUFA:SFA ratio, PUFA:MUFA ratio, total omega 6 (n-6; %), total omega 3 (n-3; %), and n-6:n-3 ratio in egg yolk of Japanese quail fed diets supplemented with different levels of *Schizochytrium* sp.

| Total fatty acids and their relationships | Microalgae <i>Schizochytrium</i> sp. (g/kg) |          |          |          |         | P-value | SEM  |
|---|---|----------|----------|----------|---------|---------|------|
|   | 0   | 10       | 20       | 30       | 40      |         |      |
| SFA*                                      | 47.900a                                     | 45.737ab | 45.467ab | 42.833ab | 41.100b | 0.04    | 1.38 |
| MUFA                                      | 36.900                                      | 37.717   | 37.433   | 38.100   | 37.900  | 0.96    | 1.23 |
| PUFA*                                     | 15.600c                                     | 16.450c  | 17.233bc | 19.167ab | 20.833a | 0.00    | 0.47 |
| MUFA:SFA                                  | 0.773                                       | 0.830    | 0.830    | 0.891    | 0.923   | 0.31    | 0.05 |
| PUFA:SFA*                                 | 0.326c                                      | 0.361c   | 0.380bc  | 0.447ab  | 0.508a  | 0.00    | 0.02 |
| PUFA:MUFA*                                | 0.423b                                      | 0.437b   | 0.463ab  | 0.503ab  | 0.550a  | 0.01    | 0.02 |
| n-6*                                      | 13.867c                                     | 14.687c  | 15.233bc | 16.800ab | 17.768a | 0.00    | 0.43 |
| n-3**                                     | 1.733b                                      | 1.720b   | 2.000b   | 2.167b   | 2.933a  | 0.00    | 0.10 |
| n-6:n-3**                                 | 8.030a                                      | 8.591a   | 7.632ab  | 7.780ab  | 6.084b  | 0.01    | 0.39 |

SEM - standard error of the mean.

N = 24 yolks per treatment.

Means within the same row with different letters differ significantly by Tukey's test (P<0.05).

\* Linear effect (P<0.05).

\*\* Quadratic effect (P<0.05).

\* SFA = 47.9080 - 0.1650x, R<sup>2</sup> = 0.96 (P = 0.004); PUFA = 15.2200 + 0.1318x, R<sup>2</sup> = 0.96 (P = 0.000); PUFA:SFA = 0.3146 + 0.0045x, R<sup>2</sup> = 0.96 (P = 0.000); PUFA:MUFA = 0.4116 + 0.0032x, R<sup>2</sup> = 0.96 (P = 0.000); n-6 = 13.6880 + 0.0991x, R<sup>2</sup> = 0.97 (P = 0.000).

\*\* n-3 = 1.7480 - 0.0129x + 0.0010x<sup>2</sup>, R<sup>2</sup> = 0.97 (P = 0.003); n-6:n-3 = 8.0777 + 0.0503x - 0.0024x<sup>2</sup>, R<sup>2</sup> = 0.87 (P = 0.040).

**Table 6** - Mean values for sensory attributes of eggs from Japanese quail fed diets supplemented with different levels of *Schizochytrium* sp.

| Sensory attribute  | Microalgae <i>Schizochytrium</i> sp. (g/kg) |        |        |        |       | P-value | SEM  |
|--------------------|---|--------|--------|--------|-------|---------|------|
|                    | 0   | 10     | 20     | 30     | 40    |         |      |
| Color*             | 7.53b                                       | 7.92ab | 7.87ab | 7.98a  | 8.06a | 0.00    | 0.11 |
| Flavor             | 7.09  | 7.62   | 7.40   | 7.53   | 7.58  | 0.08    | 0.15 |
| Aroma*             | 7.23b                                       | 7.72ab | 7.51ab | 7.64ab | 7.94a | 0.00    | 0.13 |
| Global impression* | 7.21b                                       | 7.74a  | 7.58ab | 7.60ab | 7.72a | 0.03    | 0.13 |
| Purchase intention | 3.72  | 4.02   | 3.66   | 3.91   | 4.08  | 0.08    | 0.11 |

SEM - standard error of the mean.

Means within the same row with different letters differ significantly by Tukey's test (P<0.05).

\* Linear effect (P<0.05).

\* Color = 7.648 + 0.0112x, R<sup>2</sup> = 0.75 (P = 0.001); aroma = 7.336 + 0.0134x, R<sup>2</sup> = 0.65 (P = 0.000); global impression = 7.394 + 0.0089x, R<sup>2</sup> = 0.43 (P = 0.032).

possibly buy quail eggs fed rations added with 40 g of microalgae/kg, while for the other treatments 0, 10, 20, and 30 g of *Schizochytrium* sp./kg, 62, 74, 60, and 75% of the tasters rated 4 to 5, respectively.

#### 4. Discussion

The results obtained in the present study for quail egg quality parameters corroborate those of Ross and Dominy (1990), who added different levels of the algae *Spirulina platensis* to the diet of laying hens and found no difference in shell thickness or specific weight.

However, the increasing levels of algae in the feed promoted a significant increment in the yolk color. Similarly, Herber-McNeill and Van Elswyk (1998), when analyzing the effect of the addition of 2.4 and 4.8% of the microalgae *Schizochytrium* in the feed of laying hens, observed a significant difference, to a level of 1% increase in the yellow color of the yolk.

The inclusion of microalgae *Schizochytrium* sp. is seen to be beneficial, as it linearly reduced the content of saturated fatty acids in Japanese quail egg yolks. In this sense, there was a significant linear increase ( $P < 0.05$ ) in the levels of PUFA on the yolks of Japanese quail eggs, obtained from birds fed diets containing the microalgae. This corroborates the results found by Pita et al. (2006), who observed significant changes in PUFA contents in egg yolks of hens that received diets added with different sources of n-3.

In another study with layers, Gladkowski et al. (2014) observed an increase of 7.33, 4.57, and 23.91% in the levels of PUFA,  $\alpha$ -linolenic acid (ALA, 18:3), and docosahexaenoic acid (22:6, DHA), respectively, as well as the reduction of the n-6:n-3 ratio in the lipid fractions of eggs. These results were also confirmed by Herber and Van Elswyk (1996), who demonstrated that by adding seaweed and fish oil to the diet of laying hens, the fatty acid profile of the eggs is modified. The authors demonstrated that the values of n-6 PUFA decreased, and the values of n-3 were significantly increased, thus providing a better n-6:n-3 ratio.

Unlike the present study, Park et al. (2015) observed an improvement in egg production and weight in layers fed diets containing *Schizochytrium* sp. However, similarly to the results of this study, these authors also observed an improvement in the yolk color, an increase in n-3 levels, and a reduction in the n-6:n-3 ratio.

In Japanese quail eggs, the occurrence of fresh yolks produced with poor pigmentation is common, with a reduction in the color of cooked egg, the most regular form of consumption. Cooked quail eggs with slightly pigmented yolks are less attractive to the consumer, and a possible solution is the inclusion of fat-soluble dyes in the feed, which increases the deposition of carotenoids in the yolk color (Oliveira et al., 2007). In this study, there was a linear increase in yolk color with the inclusion of microalgae in quail diet.

The addition of seaweed to the diet of laying hens results in eggs with high levels of PUFA n-3; however, Stansby (1982) mentioned that high levels of these fatty acids may predispose the final product to present a strange odor and taste due to the formation of undesirable compounds. However, in this study, it was observed that the inclusion of up to 40 g of *Schizochytrium* sp./kg did not compromise the aroma or flavor of the eggs. Thus, there was a linear increase in the aroma attribute as a function of the level of inclusion of microalgae in the feed.

The tasters attributed to the egg samples, for all the sensory characteristics evaluated, a score higher than 6 ("I liked it a little"), minimum value for the sample not to be considered as "refused". This, therefore, demonstrates the acceptance of eggs fortified with n-3 from the quail fed diets containing different levels of microalgae.

#### 5. Conclusions

The inclusion of 40 g of *Schizochytrium* sp./kg in the diet of Japanese laying quail can be used without negative effects on performance or egg quality, besides promoting higher yolk color score and

improving the egg sensory attributes color, aroma, and overall impression, as well as a higher content of n-3 and an adequate n-6:n-3 fatty acid ratio.

### Conflict of Interest

The authors declare no conflict of interest.

### Author Contributions

Conceptualization: M.O. Mendonça. Data curation: L.C. Santana, M.O. Mendonça, V.R.O. Silva, M.D.A. Castro and P.K.F. Costa. Formal analysis: L.C. Santana, M.O. Mendonça, V.R.O. Silva and G.S. Moura. Funding acquisition: M.O. Mendonça and G.S. Moura. Investigation: L.C. Santana, M.O. Mendonça, M.D.A. Castro, P.K.F. Costa and G.S. Moura. Methodology: M.O. Mendonça, V.R.O. Silva, G.S. Moura and A.G. Bertechini. Project administration: L.C. Santana, M.O. Mendonça and G.S. Moura. Resources: L.C. Santana. Supervision: M.O. Mendonça, V.R.O. Silva and G.S. Moura. Validation: L.C. Santana and V.R.O. Silva. Visualization: L.C. Santana, M.O. Mendonça and V.R.O. Silva. Writing-original draft: L.C. Santana, M.O. Mendonça, V.R.O. Silva, M.D.A. Castro, P.K.F. Costa, G.S. Moura and A.G. Bertechini. Writing-review & editing: L.C. Santana, M.O. Mendonça, G.S. Moura and A.G. Bertechini.

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