

Probiotic supplementation in diets for laying hens and its effects on the internal quality of eggs stored under refrigeration

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ABSTRACT. This study aimed to evaluate probiotics supplementation in diets for semi-heavy layers hens and their effects on the internal quality of eggs stored under refrigeration for different periods. 210 Hisex Brown® laying hens aged 30 weeks were distributed in a completely randomized design with six treatments, and seven replications. The experimental diets were offered for 112 days. The experimental diets were: Control: control diet, without the inclusion of probiotics and feed efficiency-enhancing additives; CP: positive control feed with the inclusion of a feed efficiency-enhancing additive (Halquinol antibiotic inclusion at 60 g kg⁻¹); C+100: control feed with the inclusion of 100 g t⁻¹ of a product based on *Bacillus subtilis* (*Bacillus subtilis* guaranteed level 2.0*10⁶ CFU g⁻¹); C+150: feed with the inclusion of 150 g t⁻¹ of the *Bacillus subtilis*-based product; C+200: feed with the inclusion of 200 g t⁻¹ of *Bacillus subtilis*-based product; C+250: feed with the inclusion of 250 g t⁻¹ of *Bacillus subtilis*-based product. A total of 588 eggs collected in the experimental plots were used (2 eggs per plot), and storage times at room temperature were 0, 5, 10, 15, 20, 25, and 30 days and were analyzed for internal egg quality. Probiotic supplementation did not influence the internal quality of the eggs. The storage time under refrigeration influenced the percentage and quality of the eggs' internal components and weight loss, with worse results in eggs stored for longer.

Keywords: Antibiotics; *bacillus subtilis*; semi-heavy layers; internal quality of eggs.

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Introduction

In layer hen poultry farming, good production results are associated with the birds' ability to make the most of the nutritional value of the food. Ramos et al. (2011) mentioned that antibiotics, used as growth promoters, have been widely used, largely indiscriminately, in the search for high zootechnical performance.

There is a growing concern about the use of these antibiotics regarding the emergence of resistant bacterial strains and even residues found in the products.

Nunes et al. (2013) reported that some probiotics fed to laying hens can establish a symbiotic relationship in the intestinal tract and offer great benefits such as the ability to improve conversion, egg production, increase shell thickness and egg weight, and improve egg internal quality.

Probiotics are live microorganisms that, when ingested in a certain amount, improve the intestinal microbial flora by excluding pathogenic microorganisms, and stimulate the host's immunity (Rocha et al., 2010).

Therefore, the objective of this study was to evaluate the probiotics supplementation in diets for semi-heavy layers and their effects on the internal quality of eggs stored under refrigeration at different times and storage conditions.

Material and methods

The research was submitted and approved by the Ethics Committee for Animal Experimentation (CEUA), under protocol 14/2018. Two hundred and ten Hisex Brown® laying hens with an initial age of 30 weeks, and an average egg production of 95.54% were distributed in a completely randomized design with six treatments,

seven repetitions of five birds per cage, each cage representing an experimental unit. The birds received the experimental diets for 112 days and at the end of this period, the birds had 45 weeks of age. The eggs were collected for analysis of the effects of storage time and conditions on the internal quality of the eggs.

The treatments the birds received were:

Control: control diet, without the inclusion of probiotics and feed efficiency-enhancing additives;

PC: positive control feed with the inclusion of a feed efficiency-enhancing additive (Guaranteed level of Halquinol antibiotic (min) 60 g kg⁻¹);

C+100: control feed with the inclusion of 100 g t⁻¹ of a product based on *Bacillus subtilis* (*Bacillus subtilis* guaranteed level 2.0*10⁶ CFU g⁻¹);

C+150: feed with the inclusion of 150 g t⁻¹ of the *Bacillus subtilis*-based product;

C+200: feed with the inclusion of 200 g t⁻¹ of the *Bacillus subtilis*-based product;

C+250: feed with the inclusion of 250 g t⁻¹ of the *Bacillus subtilis*-based product.

Birds were housed in a shed with a ceramic tile roof, with 42 metal cages measuring 50 cm in front and 45 cm in depth, with five birds per cage, providing 450 cm² per bird, where each cage constituted an experimental unit. Cages were equipped with individual metal feeders and nipple drinkers. Water was provided *ad libitum*, and the feed used in all treatments was isonutritional, formulated following the recommendations of Rostagno et al. (2017) (Table 1).

Table 1. Calculated nutritional composition and feed formula for commercial Hisex Brown laying hens aged 30 to 45 weeks receiving different diets supplemented or not with commercial probiotics for 112 experimental days.

| Ingredients (%) | ¹ Control | ² PC | ³ C+100 | ⁴ C+150 | ⁵ C+200 | ⁶ C+250 |
|---|----------------------|-----------------|--------------------|--------------------|--------------------|--------------------|
| Corn | 60.11 | 60.105 | 60.10 | 60.095 | 60.09 | 60.085 |
| Soybean Meal (45%) | 25.62 | 25.62 | 25.62 | 25.62 | 25.62 | 25.62 |
| Laying hen mixture* | 500 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Grit calcitic limestone | 4.68 | 4.68 | 4.68 | 4.68 | 4.68 | 4.68 |
| Soybean oil | 2.15 | 2.15 | 2.15 | 2.15 | 2.15 | 2.15 |
| Dicalcium phosphate | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 | 1.15 |
| Fine calcitic limestone | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Salt | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| DL-Methionine | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Growth enhancer | - | 0.005 | - | - | - | - |
| Probiotics (kg) | - | - | 0.010 | 0.015 | 0.020 | 0.025 |
| Total (%) | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated nutritional composition | | | | | | |
| Metabolizable Energy (kcal kg ⁻¹) | 2,850.00 | 2,850.00 | 2,850.00 | 2,850.00 | 2,850.00 | 2,850.00 |
| Crude Protein (%) | 15.88 | 15.88 | 15.88 | 15.88 | 15.88 | 15.88 |
| Crude Fiber (%) | 2.94 | 2.94 | 2.94 | 2.94 | 2.94 | 2.94 |
| Calcium (%) | 3.97 | 3.97 | 3.97 | 3.97 | 3.97 | 3.97 |
| Phosphorus (%) | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 |
| Methionine (%) | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 |

*Mixture guaranteed levels per kg of product: calcium (max) 280 g, calcium (min) 240 g, phosphorus (min) 30 g, sodium (min) 25 g, methionine (min) 16 g, choline (min.) 6,000 mg, vitamin A (min.) 200,000 IU, vitamin D3 (min.) 50,000 IU, vitamin E (min.) 150 IU, vitamin K3 (min.) 50 mg, vitamin B1 (min.) 50 mg, vitamin B2 (min.) 130 mg, vitamin B6 (min.) 40 mg, vitamin B12 (min.) 360 µg, calcium pantothenate (min.) 180 mg, niacin (min.) 400 mg, biotin (min.) 2 mg, folic acid (min.) 20 mg, iron (min.) 1,000 mg, copper (min.) 600 mg, cobalt (min.) 5 mg, iodine (min.) 20 mg, manganese (min.) 1,400 mg, zinc (min.) 1,200 mg, selenium (min.) 4 mg, phytase 10,000 FTU. ¹Control: control feed, without the inclusion of probiotics; ²PC: positive control feed with the inclusion of a feed efficiency-enhancing additive (Halquinol antibiotic guaranteed levels (min.) 60g kg⁻¹); ³C+100: control feed with the inclusion of 100 g t⁻¹ of a product based on *Bacillus subtilis* (*Bacillus subtilis* guaranteed levels 2.0*10⁶ CFU g⁻¹); ⁴C+150: feed with the inclusion of 150 g t⁻¹ of *Bacillus subtilis*-based product; ⁵C+200: feed with the inclusion of 200 g t⁻¹ of *Bacillus subtilis*-based product; ⁶C+250: feed with the inclusion of 250 g t⁻¹ of *Bacillus subtilis*-based product.

Daily management throughout the experimental period consisted of collecting and counting the eggs (the number of broken, cracked, soft-shelled, and unshelled eggs were calculated daily), supplying the feed twice a day, and taking readings of the maximum and minimum temperatures, dry and wet bulb temperatures, and relative humidity (RH). The artificial lighting was controlled by an automatic clock (timer), allowing the lights to turn on and off throughout the experimental period, totaling 17 hours of photoperiod per day, following the procedure adopted in commercial farms and the recommendations of HFAC (Humane Farm Animal Care) Standards 2014/17BR for the Production of Laying Chickens, which indicate a minimum period of six hours of continuous darkness.

The storage times in the refrigerator to evaluate egg internal quality were 0, 5, 10, 15, 20, 25, and 30 days, with a sample of two eggs per experimental plot for each day of storage. A total of 588 eggs were used for the

refrigerated storage condition. For the selection of eggs, only intact eggs were considered suitable, discarding cracked eggs, those with internal cracks, two yolks, porous shells, and deformed eggs.

The collected eggs were stored on the refrigerator shelf in cardboard cartons with a capacity of 30 eggs each, taking care to place the thin end of the eggs downwards and covering the top of the eggs with another cardboard carton.

Temperature and relative humidity of the air inside the refrigerator were measured by an Instruter data-logger, model H70, every 5 minutes. The average temperature and relative humidity inside the refrigerator during the experimental period were 7.08 °C and 61.63%, respectively.

The collected eggs were identified with a pencil with the plot number and repetition on the day of collection and weighed individually on a Marte scale, model AD4200 accurate to 0.1 g, for subsequent calculation of the weight loss according to storage days.

Variables analyzed:

- Weighing of individual eggs to calculate the percentage of weight loss according to storage time;
- Assessment of the Haugh Unit (HU) using the equation $[HU = 100 \cdot \log(h + 7.57 - 1.7 \cdot W^{0.37})]$ with data on egg weight (g) and albumen height (mm), (Moura et al., 2008), which was measured using a Starrett Series 798 Digital Caliper.
- Yolk Index (GI) was calculated using the formula $[YI = h/d]$, according to Silva (2004), where h = yolk height (mm), d = yolk diameter (mm);
- Yolk color: the eggs sampled from each plot were broken and placed on a flat surface to determine their color using the Digital Yolk Color Fan device.
- Yolk weight and shell weight after drying to calculate the albumen weight to estimate the percentages of albumen, yolk, and shell;
- pH of the yolk and albumen was measured using a HANNA pH meter model HI 99163. After breaking the eggs, the yolk and albumen were weighed and separated into 50 mL disposable plastic cups, the pH meter electrode was inserted, and three readings were taken for each component evaluated. In fresh eggs, albumen pH can vary from 7.6 to 8.5, and in stored eggs, it can reach 9.7, for the yolk, the values are 6.0 and 6.4 - 6.9, respectively (Oliveira & Oliveira, 2013).

Data were tested for normality of error by the Shapiro-Wilk test. If this assumption was not met, the variable transformation $\sqrt{x}+1$ was used. The means of the treatments were compared using orthogonal contrast (5% probability) and the means of storage time data were analyzed using regression analysis in the SISVAR statistical software (Ferreira, 2019).

Results and discussion

The treatments influenced the albumen weight (g), where the orthogonal contrast between the treatment (CP) using an antibiotic growth enhancer resulted in a lower albumen weight (g) compared to the C+100 treatment ($p < 0.05$).

For orthogonal contrasts between CP and C+150 ($p < 0.01$), and CP and C+250 ($p < 0.05$), higher yolk weight (g) and yolk percentage (%) were observed in eggs from birds supplemented with 150 g t⁻¹ and 250 g t⁻¹ of probiotic than those on dietary antibiotic supplementation. According to Asli et al. (2007), there was no effect of including probiotics in the diet for 62-week-old light layers on egg quality, except for the percentage of yolk, which was higher than in the control diet. Probiotic supplementation seems to have a positive effect on eggshell percentage. Following this reasoning, Abdelqader et al. (2013); Li et al. (2006) reported a positive effect of probiotic supplementation on eggshell quality characteristics.

The orthogonal contrast between CP and C+150 ($p < 0.05$) and between CP and C+250 ($p < 0.05$) indicated that supplementation with 150 g t⁻¹ and 250 g t⁻¹ resulted in a higher percentage of albumen (%).

The orthogonal contrast between CP and Control ($p < 0.01$) and between CP and C+200 ($p < 0.01$) showed that dietary supplementation without the inclusion of probiotics and 200g t⁻¹ of probiotics presented a higher shell percentage (%). The orthogonal contrasts between CP and C+100 ($p < 0.01$), CP and C+150 ($p < 0.01$), CP and C+200 ($p < 0.01$), CP and C+250 ($p < 0.05$) demonstrated that the inclusion of probiotics at 100 g t⁻¹, 150 g t⁻¹, 200 g t⁻¹ and 250 g t⁻¹ provided better yolk color.

The treatments ($p > 0.05$) did not affect the variables eggshell weight (g), Haugh Unit, yolk index, weight loss (%), weight loss (g), albumen and yolk pH, fresh egg weight (g), and egg weight after storage (g). Giampauli et al. (2005) found no effect of including probiotics on egg specific weight and Haugh unit. On weight loss, Abdulrahim et al. (1996) and Haddadin et al. (1996) reported similar results for laying hens fed diets supplemented with probiotics because they did not detect a difference in this characteristic, as listed in Table 2.

Table 2. Storage time of eggs under refrigeration and its effects on the internal quality of eggs from commercial Hisex Brown laying hens aged 45 weeks on diets supplemented or not with commercial probiotics for an experimental period of 112 days.

| Treatments | | Probabilities | | | | | | | | | |
|------------------------------|----------------------|-----------------|---------------------|----------------------|----------------------|---------------------|--------|-------------|--------------------|---------------------|------------------|
| Variable analyzed | Control ^a | CP ^b | C+100 ^c | C+150 ^d | C+200 ^e | C+250 ^f | Treat. | Storage day | Treat* storage day | CV (%) ⁶ | SEM ⁷ |
| Albumen weight (g) | 40.59 | 41.16 | 42.52 ² | 40.64 | 40.73 | 41.06 | 0.04 | 0.28 | 0.73 | 4.02 | 0.189 |
| Yolk weight (g) | 16.10 | 15.95 | 16.39 | 16.75 ^{3**} | 16.16 | 16.66 ^{5*} | 0.03 | 0.21 | 0.81 | 4.33 | 0.080 |
| Shell weight (g) | 6.11 | 5.89 | 5.97 | 5.98 | 6.07 | 6.04 | 0.24 | 0.00 | 0.97 | 3.49 | 0.028 |
| Albumen (%) | 64.10 | 64.30 | 64.61 | 63.48 ^{3*} | 63.87 | 63.49 ^{5*} | 0.00 | 0.00 | 0.14 | 1.27 | 0.096 |
| Yolk (%) | 23.29 | 23.18 | 22.95 | 23.93 ^{3**} | 23.07 | 23.64 ^{5*} | 0.00 | 0.00 | 0.34 | 2.46 | 0.070 |
| Shell (%) | 11.84 ^{1*} | 11.52 | 11.34 | 11.53 | 11.96 ^{4**} | 11.85 | 0.00 | 0.00 | 0.73 | 2.91 | 0.041 |
| Haugh Unit (HU) | 83.76 | 84.40 | 83.88 | 82.07 | 81.48 | 85.03 | 0.09 | 0.00 | 0.94 | 4.53 | 0.406 |
| Yolk Index | 0.43 | 0.43 | 0.44 | 0.43 | 0.43 | 0.44 | 0.20 | 0.00 | 0.80 | 1.08 | 0.001 |
| Weight loss (%) | 1.05 | 1.08 | 1.11 | 1.13 | 1.23 | 1.11 | 0.60 | 0.00 | 0.55 | 9.16 | 0.029 |
| Weight loss (g) | 0.67 | 0.68 | 0.72 | 0.73 | 0.78 | 0.71 | 0.55 | 0.00 | 0.62 | 7.08 | 0.017 |
| Albumen pH | 7.88 | 7.86 | 7.91 | 7.91 | 7.89 | 7.89 | 0.73 | 0.00 | 0.00 | 1.09 | 0.011 |
| Yolk pH | 6.45 | 6.46 | 6.48 | 6.46 | 6.40 | 6.42 | 0.70 | 0.00 | 0.25 | 1.91 | 0.016 |
| Fresh egg weight (g) | 63.83 | 63.88 | 65.59 | 64.26 | 64.07 | 63.99 | 0.11 | 0.06 | 0.33 | 2.64 | 0.201 |
| Yolk color | 4.87 | 5.03 | 4.53 ^{2**} | 4.62 ^{3**} | 5.51 ^{4**} | 4.74 ^{5*} | 0.00 | 0.00 | 0.36 | 5.67 | 0.038 |
| Egg weight after storage (g) | 63.33 | 63.08 | 64.80 | 63.78 | 63.02 | 63.44 | 0.15 | 0.16 | 0.95 | 5.69 | 0.211 |

R² = coefficient of determination. ^aControl: control feed, without the inclusion of probiotics; ^bCP: positive control feed with the inclusion of a feed efficiency-enhancing additive (Halquinol antibiotic guaranteed level (min.) 60g kg⁻¹); ^cC+100: control feed with the inclusion of 100 g t⁻¹ of a product based on *Bacillus subtilis* (*Bacillus subtilis* guaranteed level 2.0*10⁶ CFU g⁻¹); ^dC+150: feed with the inclusion of 150 g t⁻¹ of *Bacillus subtilis*-based product; ^eC+200: feed with the inclusion of 200 g t⁻¹ of *Bacillus subtilis*-based product; ^fC+250: feed with the inclusion of 250 g t⁻¹ of *Bacillus subtilis*-based product; Orthogonal contrasts: ¹y = mControl - mCP, ²y = mCP - mC+100, ³y = mCP - mC+150, ⁴y = mCP - mC+200, ⁵y = mCP - mC+250. ⁶CV(%) coefficient of variation; ⁷SEM: Standard Error of the Mean. *p < 0.05; **p < 0.01.

Under refrigerated conditions, the albumen percentage (%) was influenced by storage time (p < 0.01 - linear effect) (Table 3 and Figure 1). As for the quality of eggs from commercial layers, Garcia (2010), Santos (2005), Sauveur (1993), and Scott and Silversides (2000) found that the albumen percentage regardless of the storage temperature showed a linear reduction as a function of the days of storage but in eggs stored at refrigerated temperatures, this reduction was more pronounced.

Table 3. Means of variables at different storage times and refrigerated storage conditions.

| variables | Storage times (days) | | | | | | | Equation/ R ² |
|--------------------------|----------------------|-------|-------|-------|-------|-------|-------|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 | |
| * Albumen Weight (g) | 40.91 | 40.22 | 40.29 | 40.11 | 38.96 | 38.91 | 38.40 | - |
| Yolk weight (g) | 15.78 | 15.92 | 16.72 | 16.97 | 16.80 | 17.28 | 16.94 | - |
| Shell weight (g) | 5.94 | 6.13 | 6.04 | 6.16 | 6.08 | 6.06 | 6.13 | Y = 0.0099x + 5.8663 R ² = 14.00% |
| * % albumen | 65.77 | 63.97 | 62.86 | 61.85 | 61.18 | 60.31 | 59.78 | Y = -0.0911x + 65.348 R ² = 87.04% |
| ** % yolk | 24.84 | 25.55 | 26.12 | 26.81 | 26.34 | 26.75 | 26.40 | Y = -0.0386x ² + 1.5001x + 13.381 R ² = 70.36% |
| ** % shell | 9.37 | 9.72 | 9.62 | 9.78 | 9.83 | 9.75 | 9.55 | Y = 0.0379x ² - 1.4516x + 21.127 R ² = 71.04% |
| HU | 92.27 | 62.14 | 51.69 | 45.25 | 40.34 | 32.02 | 36.00 | Y = 0.0173x ² - 0.6838x + 88.071 R ² = 27.56% |
| YI | 0.42 | 0.38 | 0.32 | 0.34 | 0.29 | 0.26 | 0.27 | Y = -0.000075x ² + 0.0017 + 0.4356 R ² = 34.42% |
| ** % weight loss | 0.00 | 0.81 | 1.53 | 2.45 | 2.92 | 3.52 | 4.25 | Y = 0.0733x + 0.0224 R ² = 96.47% |
| ** weight loss (g) | 0.00 | 0.52 | 0.98 | 1.53 | 1.86 | 2.26 | 2.73 | Y = 0.0471x + 0.0135 R ² = 96.92% |
| Albumen pH | 8.09 | 8.23 | 7.12 | 7.00 | 10.00 | 8.33 | 8.93 | Y = 0.0051x ² - 0.1494x + 8.4888 R ² = 46.42% |
| Fresh egg weight (g) | 63.35 | 63.71 | 64.30 | 64.62 | 64.34 | 64.56 | 64.22 | - |
| * Yolk color | 5.04 | 4.94 | 4.77 | 4.91 | 5.23 | 5.25 | 5.63 | Y = 0.0019x ² - 0.0515x + 5.051 R ² = 93.39% |
| Egg weight after storage | 63.35 | 63.18 | 62.98 | 63.18 | 61.92 | 62.29 | 61.52 | - |

*p < 0.05; **p < 0.01

Yolk percentage (%) was influenced by the storage time under refrigeration (p < 0.01 - quadratic effect) (Figure 2). Santos et al. (2009) obtained, at 21 days, a higher yolk percentage compared to seven and 14 days. This increase in yolk percentage was due to the transfer of water from the albumen to the yolk due to the difference in osmotic pressure caused by water loss from the albumen to the external environment through the pores in the shell. The loss of CO₂ along storage causes the albumen protein to break down, generating water that enters the yolk through the passage from the less concentrated medium (albumen) to the more

concentrated medium (yolk). This loss is associated with evaporation that occurs through the loss of water through the pores of the shell, which also directly determines the increase in the size of the air chamber, in which the speed of moisture loss to the environment is related to the shell porosity and environmental factors such as relative air humidity, which is extremely important as an influence on egg weight loss, as it can lead to an intensification of this loss of egg weight during storage, that is, the higher the humidity, the lower the weight decline (Figueiredo et al., 2011).

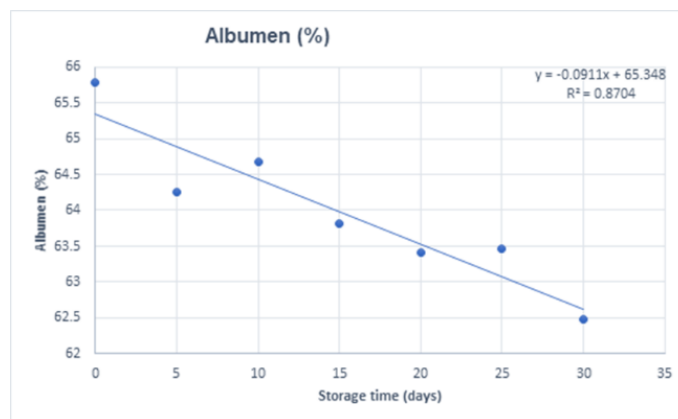


Figure 1. Influence of storage time (in days) of eggs under refrigeration on albumen percentage.



Figure 2. Influence of storage time (in days) of eggs under refrigeration on yolk percentage.

Shell percentage (%) was influenced by the storage time under refrigeration ($p < 0.01$ - quadratic effect) (Figure 3). Likewise, Figueiredo et al. (2011); Garcia et al. (2010) and Santos et al. (2009) observed a greater proportion of eggshells as the storage period increased. According to these authors, the result was a reflection of the lower weight loss of eggs kept under refrigeration, meaning that the shell weight did not increase its proportion to the total egg weight.

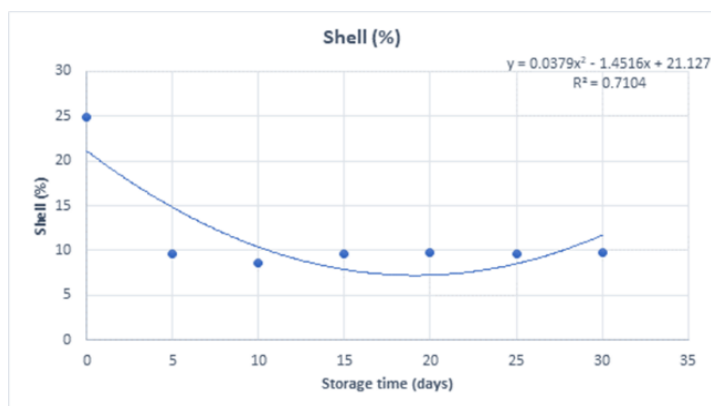


Figure 3. Influence of storage time (in days) of eggs under refrigeration on shell percentage.

Yolk color was affected by the storage time under refrigeration ($p < 0.01$ - quadratic effect) (Figure 4). Andrade et al. (2009) stated that refrigerated storage does not affect yolk color but they observed that eggs stored for longer periods, at room temperature, had a decrease in tone.

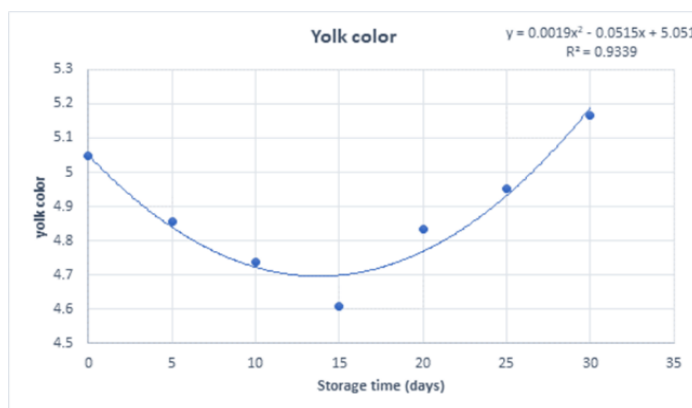


Figure 4. Influence of storage time (in days) of eggs under refrigeration on yolk color.

The variables with a significant effect from storage time were weight loss in grams (g) ($p < 0.01$ -quadratic effect) (Figure 5), and the percentage of weight loss (%) ($p < 0.01$ - linear effect) (Figure 6). This was probably due to the transfer of moisture from the albumen to the external environment through the shell, caused by the storage time of the eggs. Similar results were reported by Barbosa et al. (2008); Figueiredo et al. (2011); Garcia et al. (2010); Jones & Musgrove (2005); Lopes et al. (2012); Samli et al. (2005), and Silversides & Scoot (2001).

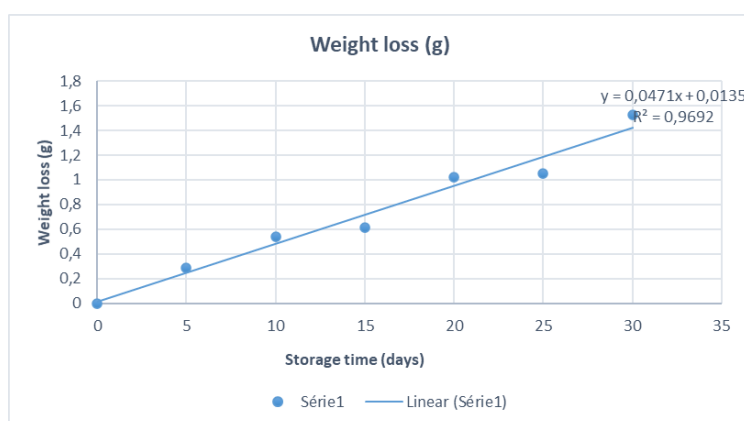


Figure 5. Influence of storage time (in days) of eggs under refrigeration on weight loss (g).

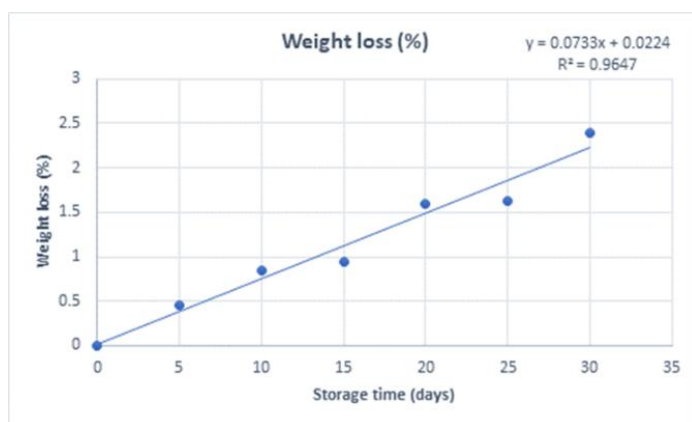


Figure 6. Influence of storage time (in days) of eggs under refrigeration on weight loss (%).

Shell weight (g) (Figure 7), Haugh Unit (HU)(Figure 8), Yolk Index (YI) (Figure 9), albumen pH (Figure 10), and yolk pH (Figure 11) were significantly ($p < 0.01$) influenced by storage days but the value for the equation was low for both linear and quadratic effects, where the values for storage days were scattered across the graph.

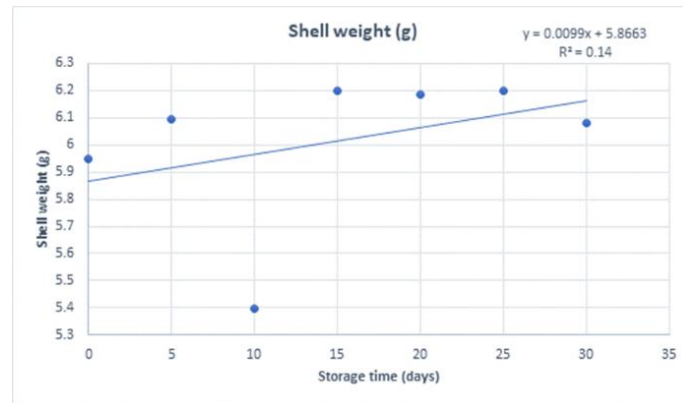


Figure 7. Influence of storage time (in days) of eggs under refrigeration on shell weight (g).

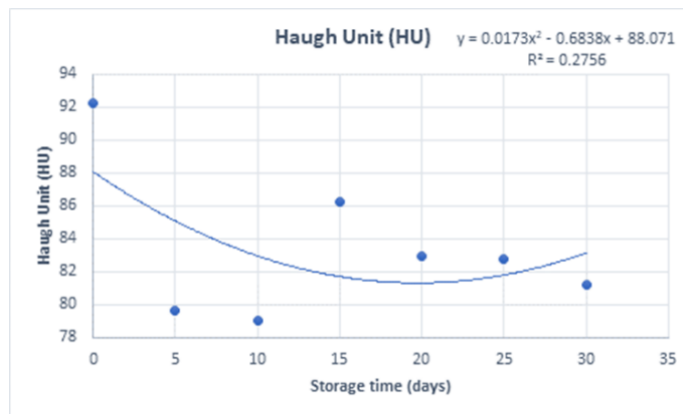


Figure 8. Influence of storage time (in days) of eggs under refrigeration on Haugh Unit (HU).

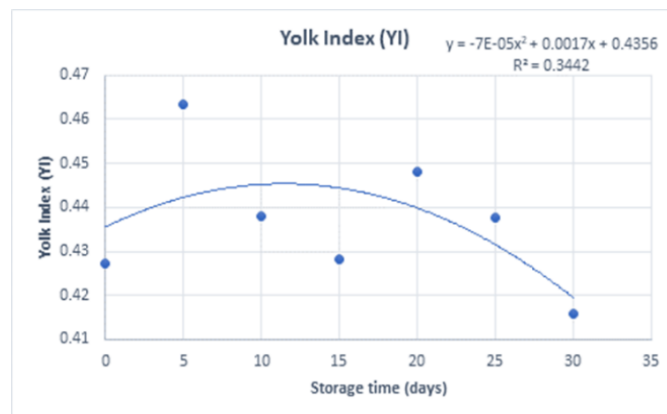


Figure 9. Influence of storage time (in days) of eggs under refrigeration on Yolk Index (YI).

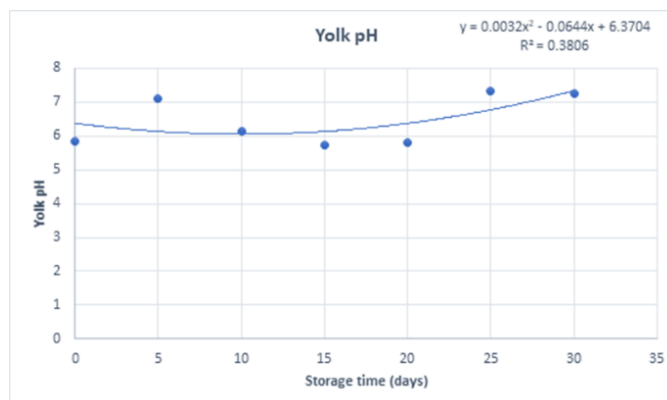


Figure 10. Influence of storage time (in days) of eggs under refrigeration on albumen pH.

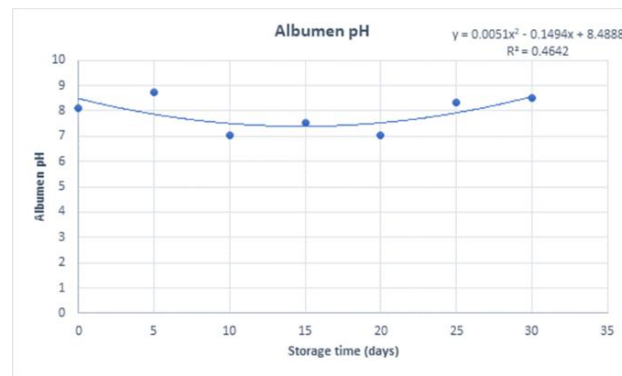


Figure 11. Influence of storage time (in days) of eggs under refrigeration on yolk pH.

Storage time did not influence and temperature conditions did not influence these variables ($p > 0.05$) albumen weight (Figure 12) and yolk weight (g) (Figure 13), fresh egg weight (g) (Figure 14), and egg weight after storage (g) (Figure 15).

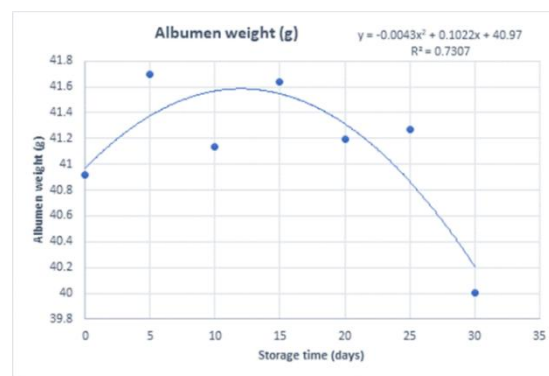


Figure 12. Influence of storage time (in days) of eggs under refrigeration on albumen weight (g).

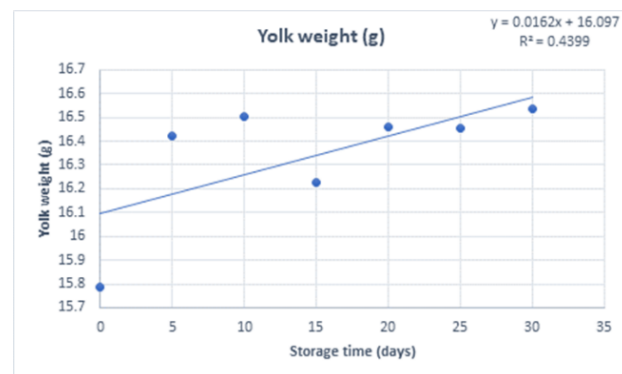


Figure 13. Influence of storage time (in days) of eggs under refrigeration on yolk weight (g).

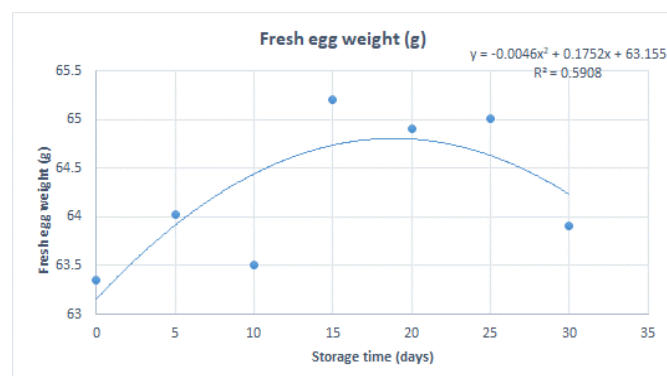


Figure 14. Influence of storage time (in days) of eggs under refrigeration on albumen weight (g).

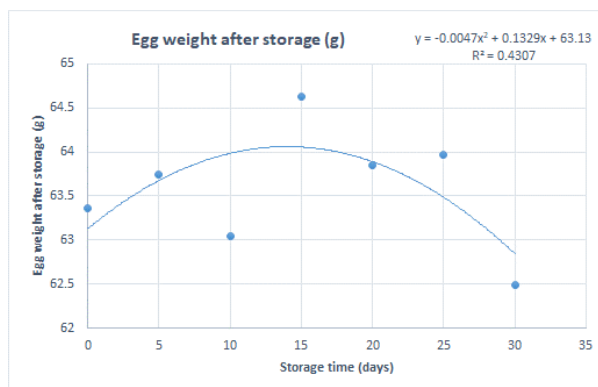


Figure 15. Influence of storage time (in days) of eggs under refrigeration on egg weight after storage (g)

Conclusion

Supplementation or not with probiotics or antibiotics does not influence the internal quality of eggs under refrigeration. The storage time under refrigeration influences the percentage and quality of egg internal components and weight loss, with worse results in eggs stored for longer.

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