ANIMAL SCIENCE

The addition of green propolis to laying hens had positive effects on egg quality: lower bacteria counts in the shell and lipid peroxidation in the yolk

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Abstract: This study aimed to determine whether the addition of green propolis extract to the diet of laying hens would improve egg quality and bird performance and decrease bacterial contamination of eggs. Forty-five brown Hy-line laying hens were used, divided into five groups with three replicates each and three animals per cage: T0 - diet without propolis; T5 - 5 grams of propolis per kg of feed; T10 - 10 grams of propolis per kg of feed; T20 - 20 grams of propolis per kg of feed and T30 - 30 grams of propolis per kg of feed. The quality of fresh eggs was made on day 21 of the experiment, and eggs were stored for 21 days. Greater specific gravity was observed in fresh eggs in T5 birds and stored eggs for T10. TBARS in fresh eggs, we found that T30 eggs had lower levels compared to other treatments. T20 eggs had the lowest total bacterial count and the lowest total coliform count in the eggshells of T10 and T20 chickens. The count of E. coli in the peel was lower for T20 and T30 than T0 and T5. The consumption of propolis did not interfere with the hens’ egg production rate but reduced food intake and consequently reduced feed conversion. We concluded that the addition of green propolis in the diet of laying birds proved efficient in reducing bacterial contamination in the eggshells and reducing the lipid peroxidation of fresh and stored eggs.

Key words: antioxidants, antimicrobial action, oxidations, propolis.

INTRODUCTION

The demand for eggs with quality and safety certifications has grown, primarily due to their high nutritional value, which contributes to the growth of laying poultry. Brazil is the fifth-largest world producer of eggs (Procópio & Lima 2020); also, it is the fifth most-consumed protein in the world. In 2019, Brazil produced around four billion dozens, of which 99.59% remained on the domestic market (ABPA 2019), with an increase in per capita egg consumption from 148 in 2010 to 230 in 2019 (ABPA 2020).

Eggs are considered foods of the highest nutritional value, as they are an excellent source of fatty acids, vitamins, minerals, and essential amino acids (Mendonça et al. 2019). However, how eggs are preserved, the composition of the diet provided to birds, and storage at room temperature, influence the internal quality of this product, as they directly affect the lipid stability of the yolk (Ding et al. 2017), as well as high temperatures, cause oxidation (Zhu et al. 2015). However, in addition to changes that may involve the physical-chemical integrity of eggs,
microbiological problems can also affect this product and pose a threat to human health. In this context, eggs are liable to be contaminated by bacteria, such as *Salmonella* spp. and *Escherichia coli*, in the bark externally by the environment (Gantois et al. 2009), or internally by a vertical transmission in which the bacteria infect the bird’s oviduct and starts to contaminate yolk, albumen, bark membranes, even before of posture (Gast et al. 2013). In this way, the use of phytogenic additives in the laying diet can provide a better physical, chemical, and biological quality of fresh and stored eggs without compromising these animals’ performance (Santos et al. 2005, Valentim et al. 2018). They can also improve the palatability of the feed, stimulate the immune system, and have anti-inflammatory, antioxidant, and antimicrobial activity (Garcés et al. 2018).

Supplementation with apicultural origin substances appears alternative in animal nutrition due to their therapeutic and prophylactic properties, with great emphasis on propolis, which has anti-inflammatory, antioxidant, immunomodulatory, analgesic, antibacterial, and antiviral activity (Genova et al. 2020). The addition of propolis to the broiler diet can stimulate the immune system and improve animal performance (Cardozo et al. 2013). Because it does not present residues, it becomes a safe additive for animal production. Thus, this study’s objective was to evaluate whether the addition of green propolis extract to the laying hens’ diet can improve animal performance, egg quality, and reduce bacterial contamination in the egg.

**MATERIALS AND METHODS**

**Propolis**

In this study, green propolis was purchased from a company located in the Chapecó region, Santa Catarina, Brazil. Propolis analysis was previously published when provided in the lamb diet, where it was recorded that the concentration of total phenolic compounds was $916.28 \pm 23.22$ mg equivalent in gallic acid/mL; the antioxidant activity ($IC_{50}$) was $158.15 \pm 4.47$ µg/mL; and the propolis extract had concentrations of gallic acid of $0.57$ mg/mL, p-coumaric acid of $3.10$ mg/mL, and chlorogenic acid of $1.41$ mg/mL (Cecere et al. 2021).

Propolis did not replace any dietary ingredient, it was an additive, that is, an extra product in the diet of hens.

**Experimental design**

This study was approved by the Committee on Ethics of Animal Welfare (CEUA) of the State University of Santa Catarina (UDESC). The experiment was conducted in the experimental shed at the State University of Santa Catarina-UDESC, located in Chapecó, Brazil. The experimental design was completely randomized, and each repetition was considered an experimental unit. The experimental period was 21 days, which corresponded to a productive cycle. Forty-five brown Hy-line laying hens, 100 weeks old, were placed in cages and divided into five groups with three replicates each, in which three animals were distributed per cage. Water and feed were ad libitum. According to the Brazilian Tables’ nutritional requirements for Poultry and Swine, the light program used natural and artificial light, which totaled 16 hours of light and eight hours of darkness, according to the Brazilian Tables’ nutritional requirements for Poultry and Swine (Rostagno et al. 2017, Table I). To stipulate consumption, the lineage manual was used, being calculated 120 g/chicken/day. All groups received the same basal diet. What changed was the addition or not of propolis. The experimental design was as follows: Group T0 birds that received only a basal diet, that is,
without propolis; group T5 birds that received 5 grams of propolis per kg of feed; T10 birds that received 10 grams of propolis per kg of feed; T20 birds that received 20 grams of propolis per kg of feed and T30 birds that received 30 grams of propolis per kg of feed.

**Animal performance**

Egg production (%): Daily, the eggs of each repetition were collected and counted. Thus, in the end, it was possible to calculate egg production (%). For the egg mass variable (g/bird/day): The eggs were identified and weighed individually for three consecutive days. Based on these data, the egg mass was obtained by the following formula: Egg mass = average weight (g) x percentage of egg production (%). As for feed consumption (g/bird/day): The rations were weighed at the beginning and at the end of the experiment to assess consumption. Feed conversion (kg/kg and kg/dozen): Feed the average feed consumption obtained conversion (AC) per kg of the egg in the experimental period divided by the egg mass,
that is, the amount of feed consumed to produce a kilogram of eggs. To calculate the feed conversion per dozen eggs produced, the amount of food consumed in the period was divided by the number of eggs produced multiplied by 12.

Analysis of egg quality
To evaluate the eggs’ quality, a sample made up of two eggs per repetition of each group was collected on the 21st and processed on the same day (fresh eggs). Two eggs per repetition of each group were collected on day 20 and stored in a temperature-controlled place (25 ± 2 °C) for 21 days. The following parameters were determined for both fresh and stored eggs: the eggs were weighed in air and water, with the value obtained, the specific gravity was calculated according to the method of Freitas et al. (2004). Eggshell strength (kgf) was measured using a Texture Analyzer (TA. XT plus) coupled to a specific eggshell test (SMS P75) adjusted with a pre-test speed of 5.0 mm/s; test speed 1.0 mm/s, and post-test speed 20 mm/s, which registered the force required to break the shell. After determining the albumen height (H) with an adapted micrometer and egg weight (W), the Haugh unit (HU) was calculated using the equation: HU = 100 log (H (mm) + 7.57 – 1.7 W (0.37 g), according to the technique described by Haugh (1937).

The yolk index (YI) was estimated with the aid of an adapted micrometer with a relation between the height (mm) and diameter of the yolk (mm). The color of the yolk was estimated using a colorimetric matrix (DSM-Yolk Color Fan®) and a colorimeter (Minolta CR-400), which determined the luminosity (L*), red intensity (a*), and intensity of yellow (b*). The shells were washed and dried at room temperature to analyze shell thickness with a digital caliper’s aid. The results were expressed as the average of the three-point measurement on the egg (apical, basal, and equatorial). Weighing of the shell, yolks, and albumen was performed to calculate the shell, yolk, and albumen percentage concerning the egg weight. The pH of the yolk and albumen were obtained using a digital pH meter (Texto-2005).

Lipid peroxidation was determined according to the methodology described by Giampietro et al. (2008), which measured substances reactive to thiobarbituric acid (TBARS) in the egg yolk. The decomposition of lipid peroxides was measured using a spectrophotometer (532 nm) and 1,1,3,3 tetramethoxyprop (TMP) as standard. The results were expressed in mg TPM/kg of yolk. The total antioxidant capacity (TAC) was evaluated as described by Erel (2004) after some changes concerning the reaction time and wavelength. TAC levels were measured on a BS 380 automated Mindray® (Shenzhen, China), and the results were expressed as μmol Trolox equivalent g⁻¹.

Bacterial counts in eggs
Egg samples (n = 6; two eggs per repeat), collected on day 22 to perform total bacterial counts (CBT) using counting agar plates, as described by Parisi et al. (2015). E. coli and total coliform counts were quantified using 3M Petrifilm EC plates. Whole shelled eggs were analyzed without cleaning. Whole shells were broken, and the internal contents were discarded. The shells were placed in sterile beakers, ground, and homogenized with a sterile glass stick. One gram of shell was diluted in 9 ml of buffered water solution with 10⁻¹ dilution for eggs. Then, 1 ml of the 10⁻¹ dilution (eggs) of each sample was inoculated into 3M Petrifilm plates for six TBC, and E. coli (EC) and 100 μL were inoculated into counting agar plates, followed by incubation for 24 48 hours at 37 °C. The results were expressed as colony-forming units per g (CFU/g).
Blood collection
Blood collection was performed on three animals per repetition, which totaled six animals per treatment, in two moments: the beginning of the experiment (day 0) and the end of the cycle (day 21 of the experiment). The animals were contained manually and using an insulin syringe; blood was collected from the ulnar vein. The blood was stored in tubes without anticoagulant to obtain the serum after centrifugation (3500 rpm for 10 min) and stored at −20 ºC until analysis.

Biochemical analyses
The serum levels of triglycerides, cholesterol, uric acid, total protein, and albumin were measured using commercial colorimetric kits (Analisa®) in the semi-automatic BioPlus 2000® equipment. Globulin levels were obtained using the mathematical formula: total protein − albumin.

Statistical analysis
The data were submitted to descriptive analysis, followed by the normality test by Shapiro–Wilk. The data without normal distribution (biochemical variables) were logged. The data were submitted to analysis of variance followed by the Tukey test. It was considered significant when P < 0.05.

RESULTS
For specific gravity, a difference was observed for fresh eggs in which the T5 treatment was superior concerning the others (P < 0.001). For the specific gravity of the stored eggs, the treatment T10 was higher (P < 0.017), while T20 was higher than the groups T5 and T30 (P < 0.017; Table II). For red intensity in both fresh and stored eggs, it was found that treatments T10, T20, and T30 were superior to treatments T5 and T0 (P < 0.001; Table II).

As for the albumen pH, a statistical difference was found for fresh eggs, in which the treatments T5 and T10 were superior to T20 and T30 (P < 0.001; Table II). As for % for the yolk, fresh eggs from T30 showed a higher value compared to treatment T5 (P < 0.050). As for the stored eggs, a difference was observed for the % of the shell, in which T5 was lower than T0, T20, and T30 (P < 0.001; Table II).

The T30 treatment showed the lowest levels of TBARS in fresh eggs compared to T0, T5, and T20 (P < 0.001). Meanwhile, in stored eggs, T30 was lower than T10 (P < 0.010). ACAP in fresh eggs T10 and T20 were superior to T0 and T5 (P < 0.001). For the total bacterial count (TBC), T20 had a lower count than the other treatments (P < 0.001). The treatments T10 and T20 had a lower total coliform count (TC) compared to the rest of the treatments (P < 0.001). For the count of E. coli in the peel, the T20 treatment had the lowest count to the T0, T5, and T10 (P < 0.001; Table II). No differences were observed regarding shell resistance, luminosity (L), yellow intensity (b), shell thickness, yolk pH and albumen (stored eggs), YI, yolk percentage, albumen, and shell (P > 0.05; Table II).

For biochemical parameters, there was no difference between groups for albumin and uric acid. However, total protein levels at 21 days decreased in all treatments than T0 (P < 0.001). Triglyceride levels at 21 days were lower at T5 compared to other treatments (P < 0.001). At 21 days at T5 and T20, cholesterol levels were lower than the others (P < 0.010). For globulins at 21 days, all treatments decreased to T0 (P < 0.001; Table III).

Feed intake g/chicken/day in treatments T5, T10, T20, and T30 were lower than T0 (P < 0.001). As for the feed conversion kg/kg of eggs, T5 showed lower conversion than the other
Table II. Egg quality of fresh eggs (days 21) and stocked eggs by 21 days from laying hens fed with differing propolis levels.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Eggs</th>
<th>T0</th>
<th>T5</th>
<th>T10</th>
<th>T20</th>
<th>T30</th>
<th>P-value</th>
<th>CV(%)</th>
</tr>
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<tbody>
<tr>
<td>Calculated gravity</td>
<td>Fresh</td>
<td>1.091&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.343&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.093&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.098&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.044&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.001</td>
<td>1.41</td>
</tr>
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<td></td>
<td>Stocked</td>
<td>1.004&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.009&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.029&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.019&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.004&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.017</td>
<td>0.98</td>
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<td>Bark resistance</td>
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<td>3779</td>
<td>3576</td>
<td>4045</td>
<td>3930</td>
<td>0.210</td>
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<td>3021</td>
<td>4027</td>
<td>4021</td>
<td>3595</td>
<td>0.064</td>
<td>30.7</td>
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<td>Color (sub)</td>
<td>Fresh</td>
<td>6.5</td>
<td>7.0</td>
<td>6.25</td>
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<td>6.25</td>
<td>0.745</td>
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<td>6.25</td>
<td>0.398</td>
<td>4.62</td>
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<td>L</td>
<td>Fresh</td>
<td>59.7</td>
<td>56.1</td>
<td>57.2</td>
<td>60.1</td>
<td>60.6</td>
<td>0.541</td>
<td>6.41</td>
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<td>49.9</td>
<td>53.0</td>
<td>56.6</td>
<td>53.7</td>
<td>50.3</td>
<td>0.056</td>
<td>7.04</td>
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<tr>
<td>A</td>
<td>Fresh</td>
<td>-5.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-5.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-6.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.11&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>-3.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-3.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-4.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-5.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-5.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.001</td>
<td>2.08</td>
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<td>B</td>
<td>Fresh</td>
<td>48.3</td>
<td>48.4</td>
<td>43.9</td>
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<td>43.1</td>
<td>0.147</td>
<td>5.89</td>
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<td>53.0</td>
<td>56.6</td>
<td>53.7</td>
<td>50.3</td>
<td>0.095</td>
<td>6.00</td>
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<td>0.255</td>
<td>0.255</td>
<td>0.254</td>
<td>0.254</td>
<td>0.954</td>
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<tr>
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<td>0.254</td>
<td>0.254</td>
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<td>0.255</td>
<td>0.991</td>
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<td>5.90</td>
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<td>6.43</td>
<td>6.12</td>
<td>6.39</td>
<td>6.17</td>
<td>6.12</td>
<td>0.697</td>
<td>1.97</td>
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<tr>
<td>pH albumen</td>
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<td>8.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
<td>0.85</td>
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<td>Stocked</td>
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<td>9.14</td>
<td>8.92</td>
<td>9.14</td>
<td>9.21</td>
<td>0.847</td>
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<td>0.445</td>
<td>0.403</td>
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<td>0.455</td>
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<td>0.199</td>
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<td>0.230</td>
<td>0.369</td>
<td>6.74</td>
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<tr>
<td>Yolk (%)</td>
<td>Fresh</td>
<td>25.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>24.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>25.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>27.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>28.5</td>
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<td>69.1</td>
<td>66.1</td>
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<td>8.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.29&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>9.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.001</td>
<td>4.06</td>
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<td>TBARS in the yolk (nmol)</td>
<td>Fresh</td>
<td>14.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>10.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>11.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.001</td>
<td>9.74</td>
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<td>14.5&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>MDA/g</td>
<td>Fresh</td>
<td>0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70</td>
<td>0.001</td>
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<td></td>
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<td>0.99</td>
<td>1.01</td>
<td>1.02</td>
<td>1.12</td>
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<td>8.62</td>
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<tr>
<td>ACAP in yolk (U.F/mg protein)</td>
<td>Fresh</td>
<td>0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70</td>
<td>0.001</td>
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<td></td>
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<td>0.99</td>
<td>1.01</td>
<td>1.02</td>
<td>1.12</td>
<td>0.369</td>
<td>8.62</td>
</tr>
<tr>
<td>TBC in shell (x10&lt;sup&gt;1&lt;/sup&gt; CFU)</td>
<td>Fresh</td>
<td>378&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>480&lt;sup&gt;a&lt;/sup&gt;</td>
<td>263&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>209&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>313&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td>Stocked</td>
<td>201&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>122&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
<td>11.3</td>
</tr>
<tr>
<td>TC in the shell (x10&lt;sup&gt;1&lt;/sup&gt; CFU)</td>
<td>Fresh</td>
<td>82.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.001</td>
<td>5.74</td>
</tr>
</tbody>
</table>

* P< 0.05 and different letters (a, b and c) on the same line show a significant difference between groups.

TBC = total bacterial cont; TC = total coliforms; E. coli = Escherichia coli. Group T0 birds that received a diet without propolis; group T5 birds that received 5 grams of propolis per kg of feed; T10 birds that received 10 grams of propolis per kg of feed; T20 birds that received 20 grams of propolis per kg of feed and T30 birds that received 30 grams of propolis per kg of feed.
treatments (P < 0.05). However, for the conversion kg/dozen eggs, T5 and T30 were similar with a lower conversion than the other treatments (P < 0.05). When measuring egg mass g/chicken/day, T5 and T30 also showed lower results than treatments (P < 0.05; Figure 1).

**DISCUSSION**

The increase in specific gravity in fresh and stored eggs seems to be related to the albumen pH which, remained lower in treatments with 5 mg/kg and 10 mg/kg of propolis, a result not observed in other studies that used this additive (Abdel-Kareem & El-Sheikh 2017, Belloni et al. 2015, Galal et al. 2008), similar to that observed by Dos Santos et al. (2020), who used a plant extract. This increase in gravity may have occurred due to less condensation of water through the shell’s pores. The effect was natural and gradual after the egg had contact with the external environment (Dos Santos et al. 2009). In this way, the decrease in pH contributed. A smaller amount of hydrogen was available to carry out the condensation and water release reaction, making the air chamber remain small, which is beneficial.

In relation, the intensity of red (a*) the eggs of treatments T10, T20, and T30 have the highest negative values for the color yolk, in which they leave a red (positive) to a green (negative) angle. This probably occurred due to the higher dosage of green propolis in the diet, which negatively influenced the yolk red intensity.

An increase in shell thickness usually occurs by depositing minerals in this structure. Propolis increases calcium and phosphorus levels in the blood plasma and their absorption by the bark

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day</th>
<th>T0</th>
<th>T5</th>
<th>T10</th>
<th>T20</th>
<th>T30</th>
<th>P-value</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total proteins (g/dL)</td>
<td>0</td>
<td>6.20</td>
<td>6.20</td>
<td>6.70</td>
<td>6.10</td>
<td>7.05</td>
<td>0.541</td>
<td>9.74</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>7.60*</td>
<td>4.90*</td>
<td>5.70*</td>
<td>5.8*</td>
<td>5.0*</td>
<td>0.001</td>
<td>5.62</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>0</td>
<td>600</td>
<td>612</td>
<td>714</td>
<td>759</td>
<td>732</td>
<td>0.169</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>803*</td>
<td>425*</td>
<td>773*</td>
<td>780*</td>
<td>751*</td>
<td>0.001</td>
<td>18.2</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>0</td>
<td>125</td>
<td>80</td>
<td>110</td>
<td>103</td>
<td>92</td>
<td>0.324</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>125*</td>
<td>88</td>
<td>162*</td>
<td>111*</td>
<td>87</td>
<td>0.010</td>
<td>9.97</td>
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<tr>
<td>Uric acid (mg/dL)</td>
<td>0</td>
<td>3.90</td>
<td>3.35</td>
<td>2.90</td>
<td>3.30</td>
<td>2.85</td>
<td>0.08</td>
<td>5.74</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>3.30</td>
<td>4.30</td>
<td>2.6</td>
<td>2.0</td>
<td>3.4</td>
<td>0.061</td>
<td>8.95</td>
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<tr>
<td>Albumin (g/dL)</td>
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<td>0.80</td>
<td>0.90</td>
<td>0.70</td>
<td>0.8</td>
<td>1.2</td>
<td>0.254</td>
<td>6.45</td>
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<tr>
<td></td>
<td>21</td>
<td>2.60</td>
<td>2.70</td>
<td>1.90</td>
<td>3.0</td>
<td>3.25</td>
<td>0.074</td>
<td>7.71</td>
</tr>
<tr>
<td>Globulins (g/dL)</td>
<td>0</td>
<td>5.40</td>
<td>5.05</td>
<td>6.0</td>
<td>6.0</td>
<td>6.2</td>
<td>0.064</td>
<td>6.78</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>5.50*</td>
<td>4.40*</td>
<td>4.15*</td>
<td>2.45*</td>
<td>2.05*</td>
<td>0.001</td>
<td>4.06</td>
</tr>
</tbody>
</table>

* P< 0.05 and different letters (a, b and c) on the same line show a significant difference between groups. Group T0 birds that received a diet without propolis; group T5 birds that received 5 grams of propolis per kg of feed; T10 birds that received 10 grams of propolis per kg of feed; T20 birds that received 20 grams of propolis per kg of feed and T30 birds that received 30 grams of propolis per kg of feed.
gland (Abdel-Kareem & El-Sheikh 2017, Seven et al. 2011). This calcium may have been directed from the bones to the shell because propolis has bone-stimulating properties (Al-Molla et al. 2014, Pereira et al. 2018).

In this study, there was a decrease in feed consumption in treatments added to propolis. Belloni et al. (2015) also observed decreased consumption when 2% and 3% of propolis were added to laying diets due to propolis’s astringent flavor. Due to its antioxidant and antibacterial properties, propolis can improve the small intestine mucosa (Prakatur et al. 2019, Xue et al. 2019). Consequently, it can improve the digestion and absorption of nutrients, which explains the lower consumption and the lower

![Bar charts showing the effects of propolis on laying rate, intake feed, feed conversion, and egg mass.](image)

**Figure 1.** Productive brown Hy-line performance laying hens, 100 weeks old supplemented with increasing propolis levels: laying rate (a), intake feed (b), feed conversion - kg/kg (c), feed conversion - kg/dz (d), and eggs mass (e). *P* < 0.05 and different letters (a, b and c) on the same line show a significant difference between groups. Group T0 birds that received a diet without propolis; group T5 birds that received 5 grams of propolis per kg of feed; T10 birds that received 10 grams of propolis per kg of feed; T20 birds that received 20 grams of propolis per kg of feed and T30 birds that received 30 grams of propolis per kg of feed.
feed conversion in T5 treatments followed by T30. However, the addition of green propolis in the dosages 5 mg/kg and 30 mg/kg reduced the egg mass, an effect that is not desirable. Belloni et al. (2015) reported that 3% of propolis’s inclusion reduced the laying rate. However, this result’s explanation is still unclear due to the controversy of the benefits of adding propolis to laying birds.

The lowest level of total protein found in the birds in this research is at odds with two other studies that used propolis in a diet (Abdel-Kareem & El-Sheikh 2017, Galal et al. 2008), these associated an increase in serum protein with the improvement of digestibility conferred by this additive. It is believed that the low protein that occurred in the present research was due to the immunostimulant effect of propolis observed by Nassar et al. (2012), which could stimulate the transport of amino acids for the development of defense cells and antibodies just as there was a decrease in serum globulins in supplemented treatments, which may be related to this stimulation and synthesis, since globulins are proteins that constitute antibodies.

The decrease in cholesterol in treatments T5 and T20 can be justified by stimulation of lipase or inhibition of cholesterol biosynthesis (Albokhadaim 2015), a phenomenon that is not well understood. However, Balasubashini et al. (2003) report that diabetic rats submitted to diets with ferulic acid showed lower lipid levels, and propolis has this substance in its composition. Thus, it is believed that it has decreased the expression of genes or enzymes contributing to cholesterol synthesis.

The addition of propolis at T10, T20, and T30 dosages reduced TBARS levels in fresh eggs and T10 dosage in stored eggs. These results indicate that the green propolis extract can minimize the effects of lipid peroxidation. T10, T20, and T30 increased antioxidant capacity biomarker (ACAP) levels in the yolk. Therefore, the propolis extract may have transferred its antioxidant compounds directly or indirectly. Thus, a possible action mechanism may be due to flavonoid compounds, phenolic acid (Khan 2017), and terpenoids (Arslan & Seven 2017). In this way, these compounds with antioxidant properties prevent or reduce the formation of free radicals such as hydrogen peroxide, which cause cellular and tissue damage. These antioxidant compounds act by donating their hydroxyl groups and, thereby, reduce malonaldehyde levels since TBARS levels have been reduced (Botsoglou et al. 2005), as seen in the present study.

Lipid peroxidation is an undesirable factor in food, as it can cause a rancid taste and, in this way, reduces the sensory and nutritional quality of eggs (Olmedo et al. 2014). Haščík et al. (2014) report that propolis’s addition decreased malonaldehyde levels in broiler meat due to its antioxidant activity compounds. Thus, propolis extract’s addition had a positive effect on the internal quality of eggs by reducing lipid peroxidation and, thus, maintaining the sensory and nutritional quality of fresh eggs stored at room temperature.

The decrease in the total bacterial count, coliforms, and *E. coli* at T10 and T20 dosages is mainly associated with the antibacterial properties of propolis (Shehata et al. 2020) that work through chemical compounds and the inhibition of the bacterium’s RNA polymerase enzyme, which compromises protein transcription and synthesis (Almuhayawi 2020). This occurs due to the permeability of the bacterial membrane, which, through the cinnamic acid and benzoic acid present in the propolis composition, acidifies the medium affecting the cell homeostasis, ATP production, and biofilm (Almuhayawi 2020, Yilmaz et al. 2018).
CONCLUSION

Therefore, it is concluded that the addition of green propolis in dosages 10 and 20 mg/kg was able to reduce the total bacterial count and *E. coli* in the eggshells. Also, it reduced peroxidation, oxidation and increased the antioxidant capacity of eggs. However, these dosages reduced the egg mass, an undesirable effect for the laying poultry chain. Further studies are needed to find an ideal dosage to maintain or improve animal performance in this context.

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Araujo DN, Boiago MM and Da Silva AS: contributed to the design and implementation of the research, to the analysis of the results. Casagrande AC, Machado GC, Brunetto AL and Galli GM: participated in the execution of the experiment and collection of samples and data. Souza CF, Baldissera MD and Rosa G did the laboratory analysis. All authors discussed the results and contributed to the final manuscript.