



## Effect of hot red pepper supplementation and different storage times on hen egg quality\*

### Efecto de la suplementación con pimienta roja picante y diferentes tiempos de almacenamiento sobre la calidad de los huevos de gallina

Muhammad Fathin Hanif<sup>1</sup>, Bambang Ariyadi<sup>2</sup>, Muhlisin<sup>3</sup>, Muhammad Asyraf Al-Huda<sup>4</sup>,  
Chiyaruna Zulfa Luschandina<sup>5</sup>, Ali Agus<sup>6</sup>

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<sup>1</sup> Universitas Gadjah Mada, Faculty of Animal Science, Department of Animal Nutrition and Feed Science. Yogyakarta, Indonesia. m.fathin@mail.ugm.ac.id (<https://orcid.org/0009-0003-6892-0356>).

<sup>2</sup> Universitas Gadjah Mada, Faculty of Animal Science, Department of Animal Production. Yogyakarta, Indonesia. bambang.ariyadi@ugm.ac.id (<https://orcid.org/0000-0002-1599-0540>).

<sup>3</sup> Universitas Gadjah Mada, Faculty of Animal Science, Department of Animal Nutrition and Feed Science. Yogyakarta, Indonesia. muhlisin.fapet@ugm.ac.id (<https://orcid.org/0000-0003-3777-6761>).

<sup>4</sup> Universitas Gadjah Mada, Faculty of Animal Science, Department of Animal Production. Yogyakarta, Indonesia. muhammad.asyraf.alhuda@mail.ugm.ac.id (<https://orcid.org/0009-0007-5752-8614>).

<sup>5</sup> Universitas Gadjah Mada, Faculty of Animal Science, Department of Animal Production. Yogyakarta, Indonesia. chiyaruna.zulfa0702@mail.ugm.ac.id (<https://orcid.org/0009-0005-5125-9519>).

<sup>6</sup> Universitas Gadjah Mada, Faculty of Animal Science, Department of Animal Nutrition and Feed Science. Yogyakarta, Indonesia. aliagus@ugm.ac.id (corresponding author; <https://orcid.org/0000-0002-9591-0669>).

## Abstract

**Introduction.** Eggs are a food source of protein that easily deteriorates during storage. Supplementation of hot red chili pepper powder, rich in bioactive compounds, in diets for laying hens can improve egg quality and prevent egg deterioration during storage. **Objective.** To determine the effect of hot red pepper powder supplementation and different storage times on the physical egg quality of laying hens. **Materials and methods.** This study was conducted from January to March 2024 at the Research Barn of PT Agromix Lestari Group, Kulon Progo, Yogyakarta, Indonesia. Eighty 56-week-old Novogen laying hens ( $1681 \pm 64$  g) were assigned into four dietary treatments (four replications, five hens per replication): basal diet as control (P0); basal diet + 0.25 % hot red pepper powder (P1); basal diet + 0.5 % hot red pepper powder (P2), and basal diet + 0.75 % hot red pepper powder (P3). At the end of week 6, one egg per replicate group was collected and analyzed for physical quality in three storage treatments: fresh condition (S0), one week storage (S1) and two weeks storage (S2). Egg quality data were analyzed with a factorial experimental design ( $4 \times 3$ ) using SPSS 26 software. **Results.** Supplementation of 0.5 % hot red pepper powder significantly increased egg weight. Hot red pepper addition and treatment duration significantly affected egg yolk color. The hot red pepper treatment group showed significantly lower pH of albumen and yolk. **Conclusions.** The supplementation of hot red



pepper powder in laying hens' diets has been shown to improve egg yolk color. The inclusion of 0.5 % in the ration was able to keep the pH of yolk and albumen low.

**Keywords:** *Capsicum annuum*, feeding stuffs, egg production, food storage, laying hens.

## Resumen

**Introducción.** Los huevos son un alimento fuente de proteínas que se deteriora fácilmente durante su almacenamiento. La suplementación de polvo de pimiento rojo picante rico en compuestos bioactivos en dietas para gallinas ponedoras puede mejorar la calidad del huevo y prevenir su deterioro durante el almacenamiento. **Objetivo.** Determinar el efecto de la suplementación con pimiento rojo picante en polvo y diferentes tiempos de almacenamiento sobre la calidad física del huevo de gallinas ponedoras. **Materiales y métodos.** El estudio se llevó a cabo de enero a marzo de 2024 en el Granero de Investigación de PT Agromix Lestari Group, Kulon Progo, Yogyakarta, Indonesia. Se asignaron 80 gallinas ponedoras de 56 semanas de edad ( $1681 \pm 64$  g) de la cepa Novogen a cuatro tratamientos dietéticos (cuatro réplicas, cinco gallinas por réplica): dieta basal como control (P0); dieta basal + 0,25 % de chile rojo en polvo (P1); dieta basal + 0,5 % de chile rojo en polvo (P2), y dieta basal + 0,75 % de chile rojo en polvo (P3). Al final de la sexta semana, se recogió un huevo por grupo y se analizó su calidad física en tres tratamientos de almacenamiento: fresco (S0), una semana de almacenamiento (S1) y dos semanas de almacenamiento (S2). Los datos de calidad de los huevos se analizaron con un diseño experimental factorial ( $4 \times 3$ ) utilizando el programa SPSS 26 (Chicago, IL, USA). **Resultados.** La suplementación con un 0,5% de pimiento rojo picante en polvo aumentó significativamente el peso del huevo. La adición de pimiento rojo picante y la duración del tratamiento afectaron significativamente al color de la yema del huevo. El grupo tratado con pimiento picante mostró un pH del albumen y la yema significativamente más bajo. **Conclusiones.** Se demostró que la suplementación de pimiento rojo picante en polvo en las dietas de gallinas ponedoras mejoró el color de la yema del huevo. La inclusión de 0,5 % en la ración fue capaz de mantener bajo el pH de la yema y el albumen.

**Palabras clave:** *Capsicum annuum*, alimentos para animales, producción de huevos, almacenamiento de alimentos, gallinas ponedoras.

## Introduction

Eggs are an important food for humans because of their nutritional content and accessible price for all people (Faber et al., 2022; Walker & Baum, 2022). As a functional food, eggs are rich in nutrients such as high-quality proteins, lipids, vitamins and minerals that are essential for human health (Réhault-Godbert et al., 2019). However, the egg is a perishable food item with a limited shelf life. Prolonged storage of eggs leads to the escape of water and carbon dioxide through the pores of the shell, increasing albumen pH and causing the egg white to become less viscous (Hester, 2017). The major difference between fresh and stored eggs is in albumen pH and quality. During storage, the pH of the egg albumen increases, which is related to the deterioration of albumen quality or Haugh unit (Feddern et al., 2017; Madrigal-Portilla et al., 2023).

The supplementation of herbs in laying hens has been a subject of interest in poultry research due to its potential impact on laying performance (Balenović et al., 2018; Hosseini et al., 2023; Li et al., 2016). The inclusion of herbs in the laying hen diet is common practice aimed at improving not only the productivity of laying hens but also to improve egg quality (Hanif et al., 2023). Herbal supplementation as a source of antioxidants and

antibacterials may also inhibit egg deterioration during storage (Agma Okur & Unver Kayhan, 2019; da Rosa et al., 2020). Supplementation with hot red pepper has been shown to improve egg quality and reduce oxidation in the egg yolk of laying hens (Abou-Elkhair et al., 2018).

Hot red pepper (*Capsicum annuum*), commonly referred to as chili, is a fruit native to Latin America that has been used since ancient times as a food vegetable, flavoring ingredient, natural coloring, and in traditional medicines (Hernández-Pérez et al., 2020). The majority of bioactive compounds in plants are secondary metabolites, such as terpenoids, phenolics, glycosides and alkaloids. The composition and concentrations of these bioactive constituents vary according to their biological factors and manufacturing and storage conditions (Abd El-Hack et al., 2022). There is no research on the effect of hot red pepper powder supplementation on different storage times on egg quality. Therefore, this research aimed to determine the effect of hot red pepper powder supplementation and different storage times on the physical egg quality of laying hens. It is hypothesized that hot red pepper supplementation can improve egg quality as well as increase egg shelf life.

## Materials and methods

### General conditions

This research was conducted from January to March 2024 in the research barn of PT Agromix Lestari Group, Indonesia, located at Dusun Banaran Kidul, Kalurahan Banguncipto, Kecamatan Sentolo, Kabupaten Kulon Progo, Special Region of Yogyakarta 55664, Indonesia. Average temperature and humidity of the layer barn were recorded at 27.9 °C (range: 23.6-34.3 °C) and 86.4 relative humidity (range: 49.7-96.0 %), respectively. The experiment received prior authorization from the Ethical Commission from the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia, with no. 98/EC-FKH/Eks./2023.

### Hot red pepper powder

Hot red pepper powder was obtained from PT Ganesha Abaditama, Jakarta, Indonesia, and was tested for bioactive compounds at the Laboratorium Penelitian dan Pengujian Terpadu (LPPT), Universitas Gadjah Mada, Yogyakarta, Indonesia. Hot red pepper was analyzed for capsaicin content by using Thin Layer Chromatography (Slameto et al., 2021); total carotene by using spectrophotometric method (Peng et al., 2005); total phenolics by using spectrophotometric method (Marina & Noriham, 2014); total flavonoid by using spectrophotometric method (Al-Matani et al., 2015); tannin content by using spectrophotometric method (Haryatmi & Susilowati, 2022); alkaloid by using spectrophotometric method (Tabasum et al., 2016), and saponin content by using spectrophotometric method (Vador et al., 2012). The bioactive compound content of hot red pepper powder is presented in Table 1.

### Birds, housing and experimental diets

Eighty 56-week-old laying hens of the Novogen strain with similar body weight ( $1681 \pm 64$  g) were divided into four treatments, each with four replicates, and five hens per replicate, in a completely randomized design. The experiment was performed for six weeks (until 62 weeks of age). Experimental diets were formulated based on corn-soybean meal according to the Novogen nutritional recommendation (Table 2). The experimental groups received different levels of hot red pepper powder: T0: control (basal diet), T1: basal diet + 0.25 % hot red pepper

**Table 1.** Bioactive compound of hot red pepper powder. Yogyakarta, Indonesia. 2024.**Cuadro 1.** Compuesto bioactivo del pimiento rojo picante en polvo. Yogyakarta, Indonesia. 2024.

| Bioactive component       | Content      |
|---------------------------|--------------|
| Capsaicin (%)             | 0.16 ± 0.00  |
| Total carotene (mg/100 g) | 80.66 ± 0.49 |
| Total phenol (%)          | 5.68 ± 0.21  |
| Total flavonoid (%)       | 0.95 ± 1.16  |
| Tannin (%)                | 3.91 ± 4.17  |
| Alkaloid (%)              | 0.34 ± 0.35  |
| Saponin (%)               | 2.85 ± 3.33  |

**Table 2.** Ingredients and nutrient composition of experimental diets for laying hens. Yogyakarta, Indonesia. 2024.**Cuadro 2.** Ingredientes y composición nutricional de dietas experimentales para gallinas ponedoras. Yogyakarta, Indonesia. 2024.

| Ingredients (%)                     | Control | HRP 0.25 % | HRP 0.50 % | HRP 0.75 % |
|-------------------------------------|---------|------------|------------|------------|
| Corn                                | 56.70   | 56.70      | 56.70      | 56.70      |
| Soybean meal                        | 24.75   | 24.75      | 24.75      | 24.75      |
| Rice bran                           | 3.65    | 3.65       | 3.65       | 3.65       |
| Crude palm oil                      | 1.50    | 1.50       | 1.50       | 1.50       |
| Di-calcium phosphate                | 1.00    | 1.00       | 1.00       | 1.00       |
| Limestone                           | 10.70   | 10.70      | 10.70      | 10.70      |
| DL-methionine                       | 0.25    | 0.25       | 0.25       | 0.25       |
| L-lysine                            | 0.10    | 0.10       | 0.10       | 0.10       |
| Salt                                | 0.25    | 0.25       | 0.25       | 0.25       |
| Mineral premix <sup>1</sup>         | 0.20    | 0.20       | 0.20       | 0.20       |
| Choline chloride                    | 0.10    | 0.10       | 0.10       | 0.10       |
| Vitamin premix <sup>2</sup>         | 0.05    | 0.05       | 0.05       | 0.05       |
| Hot red pepper powder               | 0.00    | 0.25       | 0.50       | 0.75       |
| Filler                              | 0.75    | 0.50       | 0.25       | 0.00       |
| Total                               | 100     | 100        | 100        | 100        |
| <b>Calculated nutrient contents</b> |         |            |            |            |
| Dry matter (%)                      | 89.89   | 89.89      | 89.89      | 89.89      |
| Metabolizable energy                | 2817    | 2809       | 2803       | 2800       |
| Crude protein (%)                   | 18.08   | 18.10      | 18.12      | 18.14      |
| Ether extract (%)                   | 3.40    | 3.40       | 3.40       | 3.40       |
| Crude fiber (%)                     | 2.30    | 2.30       | 2.30       | 2.30       |
| Calcium (%)                         | 4.30    | 4.30       | 4.30       | 4.30       |
| Total phosphorus (%)                | 0.50    | 0.50       | 0.50       | 0.50       |
| Methionine (%)                      | 0.96    | 0.96       | 0.96       | 0.96       |
| Lysine (%)                          | 0.53    | 0.53       | 0.53       | 0.53       |

<sup>1</sup> Contained per kg: Calcium 261.5 g; sodium 29.3 g; iron 17.7 g; phosphorus 6.9 g; magnesium 2.5 g; potassium 2.4 g; manganese 900 µg; sulphur 783.6 µg; zinc 361.6 µg; copper 186.6 µg; cobalt 5.3 µg, and selenium 0.61 µg. / 1 Contenido por kg: Calcio 261,5 g; sodio 29,3 g; hierro 17,7 g; fósforo 6,9 g; magnesio 2,5 g; potasio 2,4 g; manganeso 900 µg; azufre 783,6 µg; zinc 361,6 µg; cobre 186,6 µg; cobalto 5,3 µg, y selenio 0,61 µg.

<sup>2</sup> Contained per kg: Carnitine 20.000 mg; vitamin A 20.000.000 IU; vitamin D3 4.000.000 IU; vitamin E 15.000 mg; vitamin K3 7.000 mg; vitamin B2 12.000 mg; vitamin B6 8.000 mg; vitamin B12 60 mg; Ca-D-pantothenate 20.000 mg; niacin 40.000 mg, and folic acid 500 mg. / 2 Contenido por kg: Carnitina 20 000 mg; vitamina A 20 000 000 UI; vitamina D3 4 000 000 UI; vitamina E 15 000 mg; vitamina K3 7000 mg; vitamina B2 12 000 mg; vitamina B6 8000 mg; vitamina B12 60 mg; Ca-D-pantotenato 20 000 mg; niacina 40 000 mg, y ácido fólico 500 mg.

powder, T2: basal diet + 0.5 % hot red pepper powder, T3: basal diet + 0.75 % hot red pepper powder. The treatment feed was fed for six weeks as recommended by Anas et al. (2019). The hens were housed in battery cages (22 cm in length, 35 cm in width, and 30 cm in height) equipped with trough feeders and nipple drinkers. The lighting regimen was 16 hours of continuous light per day from 06:00 to 22:00.

### Egg quality assay

In each replicate group, eggs were collected on days 0, 21 and 42 for physical quality assessment. The egg shape index was determined by calculating the ratio of vertical and horizontal diameter using the Egg Form Coefficient Measurement Instrument (FHK, Fujihira Industry Co., Ltd., Tokyo, Japan). Egg volume was measured using a 1000 ml measuring cylinder glass. Egg specific gravity was determined by dividing the weight of the egg by the volume of the egg. Eggshell straightness was measured using a pressure gauge (FHK, Fujihira Industry Co., Ltd., Tokyo, Japan). The height of the albumen was measured as the distance between the metal plate and electrode placed on top of the thickest part of the egg, while the yolk height was measured after the yolk was separated from the albumen, using a micrometer (FHK, Fujihira Industry Co., Ltd., Tokyo, Japan). The albumen index and yolk index were measured by dividing the height by the diameter. The Haugh units were assessed following equation 1 (Haugh, 1937).

$$HU = 100 \log (\text{albumen height (mm)} + 7.57) 1.7 * \text{egg weight (g)}^{0.37} \quad \text{Equation [1]}$$

The weights of the egg, eggshell, albumen and egg yolk were measured using a high-precision digital scale with a capacity of 500 g/0.01 g (PT. Arta Joil Tappa, Indonesia). Yolk color was determined using the Roche yolk color fan scores (RYCF; F. Hoffman-La Roche, Basel, Switzerland), and colors were scored according to 15 sample colors ranging from 1 (the lightest) to 15 (the darkest).

### Egg storage treatment

At the end of week 6, three eggs per replicate group were collected to be divided into three room temperature storage treatments, namely: no storage (fresh egg), one-week storage and two-week storage. The eggs were stored in the Laboratory of Poultry Science, Universitas Gadjah Mada, Indonesia, at room temperature ( $\pm 25^\circ\text{C}$ ). Subsequently, the eggs were analyzed for physical quality to determine changes in albumen index, yolk index, albumen pH, yolk pH and Haugh unit. The pH of albumen and yolk was measured using a pH meter (ATC, China).

### Statistical analysis

Egg quality data were analyzed using a  $3 \times 4$  factorial experimental design, 3 storage treatments durations and 4 dosage levels of hot red pepper powder. Egg stability data during storage were analyzed using a  $3 \times 4$  factorial experimental design, 3 storage times and 4 levels of hot red pepper powder dosage. All data were analyzed using SPSS 26 software (Chicago, IL, USA).

## Results

### Effect of level of hot red pepper on egg quality of laying hens

The hot red pepper treatment 0.75 % diet group showed higher egg specific gravity than the control group ( $p < 0.05$ , Table 3). In addition, interaction analysis showed an interaction between hot red pepper level and treatment

**Table 3.** Effect of hot red pepper supplementation on the physical quality of eggs from laying hens at weeks 0, 3 and 6. Yogyakarta, Indonesia. 2024.**Cuadro 3.** Efecto de la suplementación con pimienta roja picante sobre la calidad física de los huevos de gallinas ponedoras en las semanas 0, 3 y 6. Yogyakarta, Indonesia. 2024.

| Treatment                         | EW<br>(g) | EI    | ESG<br>(g/ml)      | ESS<br>(mPa)        | ESW<br>(%) | EST<br>(mm) | AI    | AW<br>(%) | YI                 | YW<br>(%) | YC                | HU    |
|-----------------------------------|-----------|-------|--------------------|---------------------|------------|-------------|-------|-----------|--------------------|-----------|-------------------|-------|
| <b>Hot red pepper level (%)</b>   |           |       |                    |                     |            |             |       |           |                    |           |                   |       |
| 0.00                              | 57.88     | 73.62 | 1.12               | 0.383               | 10.73      | 0.367       | 0.083 | 63.56     | 0.360              | 25.73     | 7.54 <sup>b</sup> | 83.63 |
| 0.25                              | 60.54     | 74.57 | 1.08 <sup>a</sup>  | 0.363               | 10.88      | 0.379       | 0.079 | 63.47     | 0.381              | 25.65     | 8.13 <sup>a</sup> | 82.37 |
| 0.50                              | 62.04     | 74.29 | 1.09 <sup>a</sup>  | 0.378               | 10.05      | 0.366       | 0.088 | 65.20     | 0.369              | 24.75     | 8.08 <sup>a</sup> | 82.40 |
| 0.75                              | 60.08     | 73.84 | 1.14               | 0.355               | 10.98      | 0.379       | 0.086 | 63.33     | 0.358              | 25.69     | 8.25 <sup>a</sup> | 85.16 |
| <b>Treatment duration (weeks)</b> |           |       |                    |                     |            |             |       |           |                    |           |                   |       |
| 0                                 | 60.85     | 73.58 | 1.07 <sup>a</sup>  | 0.352 <sup>a</sup>  | 10.82      | 0.381       | 0.089 | 63.71     | 0.376 <sup>b</sup> | 25.47     | 7.53 <sup>a</sup> | 83.86 |
| 3                                 | 60.35     | 73.85 | 1.14 <sup>b</sup>  | 0.361 <sup>ab</sup> | 10.76      | 0.366       | 0.085 | 63.52     | 0.378 <sup>b</sup> | 25.72     | 8.00 <sup>b</sup> | 83.82 |
| 6                                 | 59.21     | 74.80 | 1.11 <sup>ab</sup> | 0.396 <sup>b</sup>  | 10.39      | 0.371       | 0.078 | 64.43     | 0.347 <sup>a</sup> | 25.17     | 8.47 <sup>c</sup> | 82.50 |
| <b>P-Value</b>                    |           |       |                    |                     |            |             |       |           |                    |           |                   |       |
| Pepper level                      | 0.116     | 0.894 | 0.320              | 0.532               | 0.100      | 0.816       | 0.441 | 0.283     | 0.093              | 0.759     | 0.000             | 0.159 |
| Duration                          | 0.518     | 0.557 | 0.004              | 0.051               | 0.412      | 0.629       | 0.460 | 0.598     | 0.001              | 0.840     | 0.000             | 0.440 |
| Pepper level × duration           | 0.526     | 0.502 | 0.011              | 0.155               | 0.014      | 0.894       | 0.205 | 0.983     | 0.116              | 0.935     | 0.005             | 0.163 |
| SEM                               | 0.598     | 0.454 | 0.010              | 0.008               | 0.160      | 0.006       | 0.002 | 0.358     | 0.004              | 0.339     | 0.085             | 0.511 |

EW: Egg weight. EI: Egg shape index. ESG: Egg specific gravity. ESS: Eggshell strength. ESW: Eggshell weight. EST: Eggshell thickness. AI: Albumen index. AW: Albumen weight. YI: Yolk index. YW: Yolk weight. YC: Yolk color. HU: Haugh unit. / EW: Peso del huevo. EI: Índice de forma del huevo. ESG: Peso específico del huevo. ESS: Resistencia de la cáscara del huevo. ESW: Peso de la cáscara del huevo. EST: Espesor de la cáscara del huevo. AI: Índice del albumen. AW: Peso del albumen. YI: Índice de yema. YW: Peso de la yema. YC: Color de la yema. HU: Unidad Haugh.

<sup>a, b</sup> Different letters in the same column indicate significant differences ( $p < 0.05$ ). / <sup>a, b</sup> Letras diferentes en la misma columna indican diferencias significativas ( $p < 0.05$ ).

SEM: Standard error of the mean. / SEM: Error estándar de la media.

duration ( $p < 0.05$ ). There was an interaction between hot red pepper level and treatment duration ( $p < 0.05$ ). Feeding hot red pepper for six weeks decreased yolk index ( $p < 0.05$ ). The inclusion of hot red pepper powder in all treatment groups (0.25 %, 0.50 %, 0.75 %) improved egg yolk color ( $p < 0.05$ ). Moreover, the duration of hot red pepper supplementation increased egg yolk color ( $p < 0.05$ ).

### Effect of storage time on egg quality of laying hens

Hen eggs deteriorated in quality during storage (Table 4). The week-long egg storage treatment decreased albumen index and increased albumen pH ( $p < 0.05$ ). In addition, egg storage treatment also decreased yolk index ( $p < 0.05$ ). Furthermore, the supplementation of hot red pepper 0.5 % decreased yolk pH ( $p < 0.05$ ). Haugh units decreased during egg storage ( $p < 0.05$ ).

**Table 4.** Effect of hot red pepper supplementation and different storage time on the quality of laying hen eggs. Yogyakarta, Indonesia. 2024.**Cuadro 4.** Efecto de la suplementación con pimienta roja picante y diferentes tiempos de almacenamiento sobre la calidad de los huevos de gallinas ponedoras. Yogyakarta, Indonesia. 2024.

| Treatment                       | EW    | ESG   | AI                 | ApH               | YI                 | YpH               | HU                 |
|---------------------------------|-------|-------|--------------------|-------------------|--------------------|-------------------|--------------------|
| <b>Hot red pepper level (%)</b> |       |       |                    |                   |                    |                   |                    |
| 0.000                           | 58.98 | 1.11  | 0.045              | 9.10              | 0.304              | 6.34 <sup>a</sup> | 56.23              |
| 0.250                           | 59.68 | 1.09  | 0.043              | 8.98              | 0.294              | 6.32 <sup>a</sup> | 50.48              |
| 0.500                           | 60.13 | 1.12  | 0.042              | 8.88              | 0.323              | 6.07 <sup>b</sup> | 50.76              |
| 0.750                           | 60.18 | 1.11  | 0.043              | 8.93              | 0.302              | 6.03 <sup>b</sup> | 51.84              |
| <b>Storage time (weeks)</b>     |       |       |                    |                   |                    |                   |                    |
| 0                               | 59.21 | 1.11  | 0.078 <sup>b</sup> | 8.74 <sup>a</sup> | 0.347 <sup>b</sup> | 6.07              | 82.50 <sup>c</sup> |
| 1                               | 60.83 | 1.13  | 0.029 <sup>a</sup> | 9.06 <sup>b</sup> | 0.296 <sup>a</sup> | 6.23              | 41.56 <sup>b</sup> |
| 2                               | 59.16 | 1.08  | 0.024 <sup>a</sup> | 9.12 <sup>b</sup> | 0.274 <sup>a</sup> | 6.28              | 32.92 <sup>a</sup> |
| <b>P-Value</b>                  |       |       |                    |                   |                    |                   |                    |
| Pepper level                    | 0.860 | 0.563 | 0.835              | 0.072             | 0.312              | 0.007             | 0.225              |
| Storage time                    | 0.383 | 0.055 | 0.000              | 0.000             | 0.000              | 0.076             | 0.000              |
| Pepper level × storage time     | 0.958 | 0.143 | 0.879              | 0.108             | 0.579              | 0.105             | 0.271              |
| SEM                             | 0.508 | 0.009 | 0.004              | 0.040             | 0.007              | 0.043             | 3.30               |

EW: Egg weight. SG: Egg specific gravity. AI: Albumen index. ApH: Albumen pH. YI: Yolk index. YW: Yolk weight. YpH: Yolk pH. HU: Haugh unit. / EW: Peso del huevo. SG: Peso específico del huevo. AI: Índice del albumen. ApH: pH del albumen. YI: Índice de yema. YW: Peso de la yema. YpH: pH de la yema. HU: Unidad Haugh.

<sup>a, b</sup> Different letters in the same column indicate significant differences ( $p < 0.05$ ). / <sup>a, b</sup> Letras diferentes en la misma columna indican diferencias significativas ( $p < 0,05$ ).

SEM: Standard error of the mean. / SEM: Error estándar de la media.

## Discussion

This research aimed to investigate the effect of hot red pepper supplementation and different storage times on egg quality. This study showed that supplementation of hot red pepper powder for three weeks increases egg specific gravity. Egg specific gravity is a critical parameter that can be influenced by various dietary supplements. Research by Guimarães et al. (2021) demonstrated a significant interaction between turmeric powder supplementation and storage period in Japanese quails, affecting the specific gravity of eggs. Similarly, Santana Santos et al. (2019) found that cinnamon powder inclusion in the diet of laying quails improved specific gravity. The specific gravity of chicken eggs is a key parameter that reflects the density of the egg and was influenced by various factors, including the specific gravity of the chorion, internal material (ovoplasm and perivitelline fluid), chorion volume and egg volume (Xia et al., 2018). In poultry production, specific gravity is an important indicator of egg quality, as it can provide insights into factors such as shell thickness, egg freshness and internal egg composition (Abou-Elkhair et al., 2014).

The supplementation of hot red pepper powder may increase the internal egg composition Abou-Elkhair et al. (2018) who reported that 0.500 % hot pepper supplementation can increase egg weight. The beneficial effects of phytogetic feed additives on gut microbiota modulation, improved nutrient digestibility and absorption, as well as

improved ovarian characteristics, can contribute to improved health status and laying performance. These effects may also be responsible for the significant impact these additives have on laying performance (Boka et al., 2014; Saki et al., 2014). In addition, the inclusion of hot red pepper increased eggshell strength. Dietary capsaicin may positively influence mineral uptake by the intestinal segments, potentially enhancing the absorption of minerals such as calcium, zinc and iron by increasing the absorptive surface in the intestines (Alam et al., 2020). This finding indicates a potential role of capsaicin in improving mineral absorption, which could have implications for enhancing mineral uptake and eggshell quality in poultry production systems.

In this study, hot red pepper supplementation also increased egg yolk color, a significant factor in consumer egg quality (Englmaierová et al., 2014; Sünder et al., 2022). These results are similar to the findings of Hanif et al. (2024), who reported that the supplementation of pepper improves egg yolk color. The increase in egg yolk color is due to carotenoid deposition in the yolk derived from the feed (Kljak et al., 2021a; Kljak et al., 2021b). Carotenoid content is widely acknowledged to be closely associated with yolk coloration. The efficacy of carotenoid transfer to the egg yolk, and consequently its influence on yolk hue, is greatly influenced by the kind and molecular makeup of the carotenoids found in natural feed additives (Abd El-Hack et al., 2022; Saleh et al., 2021). The egg yolk color was stated to be influenced by the consumption of zeaxanthin, lutein, alpha-carotene, beta-carotene and carotenoids (Hammershøj et al., 2010).

Egg storage often leads to decline egg quality, characterized by various detrimental changes such as thinning of the albumen (Omana et al., 2011), increased pH value, rupture of the vitelline membrane, and increased water content in the yolk (Yuceer & Caner, 2014). Carbon dioxide (CO<sub>2</sub>) from the albumen can be dissipated continuously through the pores of the shell. CO<sub>2</sub> in eggs is formed when the balance of the carbonate-bicarbonate buffer system shifts towards the production of CO<sub>2</sub> in eggs (Akter et al., 2014). Loss of CO<sub>2</sub> in stored chicken eggs and quail eggs was reported by Akter et al. (2014) and Jin et al. (2011). Gelation of egg albumen is conceived as a two-step process. Firstly, some proteins are denatured, while the second step involves the aggregation of the denatured proteins. The degree of denaturation is associated with the opening of the proteins, the nature of the interaction or bonding, and the kinetics of the aggregation process, and these factors determine the type and characteristics of the resulting gel (Campbell et al., 2003).

Denaturation of some albumen proteins, such as trypsin inhibitors, occurs to varying degrees during storage of duck and chicken eggs, which may be due to differences in stability and molecular properties of proteins between the two types of eggs (Qiu et al., 2012; Quan & Benjakul, 2018). During storage, alkaline ions such as sodium, potassium, and magnesium in the egg move the albumen fluid toward the yolk, leading to an increase in yolk pH value (Thohari et al., 2022). Moreover, Wibowo and Sudjatinah (2023) explained that the pH increase in egg yolk during storage is attributed to the absorption of water from the albumen or lipid peroxidation of polyunsaturated fatty acid. This research showed that hot red pepper supplementation decreased the pH of egg albumen and yolk. Antioxidant compounds such as phenols, flavonoids and tannins may prevent oxidation in eggs (Cai et al., 2006). The supplementation of tannin-rich feed additives maintains egg quality during storage (Cornescu et al., 2022).

The bioactive compounds present in hot red pepper may contribute to maintaining the stability of egg quality from damage caused by microorganisms during storage. Hot red pepper contains bioactive compounds that can act as antibacterials (Nagy et al., 2015; Valková et al., 2021). During storage, the pores of the shell are enlarged, making it easier for microorganisms to enter and exit the shell (Eke et al., 2013). Microorganisms such as yeast and mold are commonly associated with egg spoilage, not food-borne diseases (Tomczyk et al., 2018; Tomczyk et al., 2019). The capsaicin compound in pepper shows the ability as antibacterial and antifungal (Morrine et al., 2018). Capsaicin exhibits concentration-dependent antibacterial effects through mechanisms such as osmotic stress induction, destruction of cell membrane structures, and inhibition of gene expression responsible for bacterial cell growth (Romero-Luna et al., 2023).

## Conclusions

The supplementation of hot red pepper powder in laying hens' diets has been demonstrated to improve egg specific gravity, eggshell strength and egg yolk color, as well as to reduce yolk index. Also, hot red pepper powder decreased yolk pH during storage. There were interactions between hot red pepper and storage time on eggshell weight, egg specific gravity and egg yolk color. The supplementation of about 0.500 % in the ration was able to keep the pH of yolk.

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## Interests conflict

The authors declare that there is no conflict of interest.

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