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Effect of hot red pepper supplementation and different storage time on hens egg quality*

Efecto de la suplementación con pimiento rojo picante y differentes tiempos de almacenamiento sobre la calidad de los huevos de gallina

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Abstract

Introduction. Eggs are a food source of protein that easily deteriorates during storage. Supplementation of hot red chilli 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 ± 64 g) were assigned into four dietary treatments (4 replications, 5 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 pepperpowder (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 (S3). Egg quality data were analyzed with a factorial experimental design (4 x 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 colour. 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 the 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 ± 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 (S3). Los datos de calidad de los huevos se analizaron con un diseño experimental factorial (4 x 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, gallina ponedora.

Introduction

Eggs are an important food for humans because of their nutritional content and also their 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, phenolic, 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 supplementation on different storage times on egg quality. Therefore, this research aimed to determine the effect of hot red pepper supplementation and different storage time on egg quality. 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 (RH; range: 49.7.0–96.0 %RH), 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 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 & Norilham, 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 presented in Table 1.

Birds, housing and experimental diets

Eighty 56-week-old laying hens of the Novogen strain with similar body weight (1681 ± 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 6 weeks (until 62 weeks of age). Experimental diets were formulated based on corn–soybean meal according to the Novogen nutritional recommendation (Table 3). The experimental groups received different levels of hot red pepper powder, T0: control (basal diet), T1: basal diet + 0.25 % hot red pepper powder,

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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

T2: basal diet + 0.5 % hot red pepper, T3: basal diet + 0.75 % hot red pepper powder. The treatment feed was fed for 6 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 high) 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 the equation 1 (Haugh, 1937).

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

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 weeks storage. The eggs were stored in the Laboratory of Poultry Science, Universitas Gadjah Mada, Indonesia at room temperature (±25 °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).

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Table 2. Ingredients and nutrient composition of experimental laying hens diets. 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[1]	0.20	0.20	0.20	0.20
Choline chloride	0.10	0.10	0.10	0.10
Vitamin premix[2]	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
Calculated nutrient contents				
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 phosphor (%)	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

[1] contained per kg: calcium 261.5 g; sodium 29.3 g; iron 17.7 g; phosphor 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.

[2] contained per kg: carnitine 20.000 mg; vitamin A 20.000.000 IU; vitamin D3 4.000.000 UI; 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-panthothenate 20.000 mg; niacin 40.000 mg; and folic acid 500 mg. / [2] contenidos 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.

Statistical analysis

Egg quality data were analyzed using a 3 x 4 factorial experimental design, 3 storage treatments durations and 4 dosage levels of hot red pepper powder. Egg stability data during storage was analyzed using a 3 x 4 factorial

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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 duration ($p<0.05$). Furthermore, there is an interaction between hot red pepper level and treatment duration ($p<0.05$). Feeding hot red pepper for 6 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 ($p<0.05$).

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

Treatment	EW (g)	EI	ESG (g/ml)	ESS (mPa)	ESW (%)	EST (mm)	AI	AW (%)	YI	YW (%)	YC	HU
Hot Red Pepper Level (%)												
0.00	57.88	73.62	1.12	0.383	10.73	0.367	0.083	63.56	0.360	25.73	7.54[b]	83.63
0.25	60.54	74.57	1.08[a]	0.363	10.88	0.379	0.079	63.47	0.381	25.65	8.13[a]	82.37
0.50	62.04	74.29	1.09[a]	0.378	10.05	0.366	0.088	65.20	0.369	24.75	8.08[a]	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[a]	85.16
Treatment Duration (weeks)												
0	60.85	73.58	1.07[a]	0.352[a]	10.82	0.381	0.089	63.71	0.376[b]	25.47	7.53[a]	83.86
3	60.35	73.85	1.14[b]	0.361[ab]	10.76	0.366	0.085	63.52	0.378[b]	25.72	8.00[b]	83.82
6	59.21	74.80	1.11[ab]	0.396[b]	10.39	0.371	0.078	64.43	0.347[a]	25.17	8.47[c]	82.50
P-Value												
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 X 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 strengthness, ESW: eggshell weight, EST: eggshell thickness, AI: albumen index, AW: albumen weight, YI: yolk index, YW: yolk index, 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 de albumen, AW: peso del albumen, YI: índice de yema, YW: índice de yema, YC: color de la yema, HU: unidad haugh.

[a, b] Different letters in the same column show significant differences ($p<0.05$). / [a, b] Letras diferentes en la misma columna muestran diferencias significativas ($p<0,05$).

SEM: mean standard error. / SEM: error estándar de la media.

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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 also 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 pH yolk ($p<0.05$). 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 pimiento rojo 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
Hot Red Pepper Level (%)							
0.000	58.98	1.11	0.045	9.10	0.304	6.34^{a}	56.23
0.250	59.68	1.09	0.043	8.98	0.294	6.32^{a}	50.48
0.500	60.13	1.12	0.042	8.88	0.323	6.07^{b}	50.76
0.750	60.18	1.11	0.043	8.93	0.302	6.03^{b}	51.84
Storage Time (weeks)							
0	59.21	1.11	0.078^{b}	8.74^{a}	0.347^{b}	6.07	82.50^{c}
1	60.83	1.13	0.029^{a}	9.06^{b}	0.296^{a}	6.23	41.56^{b}
2	59.16	1.08	0.024^{a}	9.12^{b}	0.274^{a}	6.28	32.92^{a}
P-Value							
Pepper Level	0.860	0.563	0.835	0.072	0.312	0007	0.225
Storage Time	0.383	0.055	0.000	0.000	0.000	0.076	0.000
Pepper Level X 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 index, YpH: yolk pH, HU: haugh unit. / EW: peso del huevo, SG: peso específico del huevo, AI: índice de albumen, ApH: pH del albumen, YI: índice de yema, YW: índice de yema, YpH: pH de yema, HU: unidad haugh.
[a, b a, b] Different letters in the same column show significant differences ($p<0.05$). / Letras diferentes en la misma columna muestran diferencias significativas ($p<0,05$).
SEM: mean standard error. / 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 3 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

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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 eggAbou-Elkhair et al. (2018) who reported that 0.500% hot pepper supplementation can increase egg weight. The beneficial effects of phytogenic 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 egg shell 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), 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 effacy 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, the increased water content in the yolk (Yuceer & Caner, 2014). Carbon dioxide (CO_2) from the albumen can be dissipated continuously through the pores of the shell. CO_2 in eggs is formed when the balance of the carbonate-bicarbonate buffer system shifts towards the production of CO_2 in eggs (Akter et al., 2014). Loss of CO_2 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 maining the stability of egg quality from damage caused by microorganisms during storage. Hot red pepper contains bioactive compounds that can

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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

In conclusion, the supplementation of hot red pepper powder in laying hens diets has been shown to improve egg specific gravity, eggshell strength, egg yolk color and decrease yolk index Moreover, hot red pepper powder decrease yolk pH during storage. Also, 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.

References

Abd El-Hack, M. E., El-Saadony, M. T., Elbestawy, A. R., Gado, A. R., Nader, M. M., Saad, A. M., El-Tahan, A. M., Taha, A. E., Salem, H. M., & El-Tarabily, K. A. (2022). Hot red pepper powder as a safe alternative to antibiotics in organic poultry feed: an updated review. *Poultry Science*, *101*(4), Article 101684. https://doi.org/10.1016/j.psj.2021.101684

Abou-Elkhair, R., Ahmed, H. A., & Selim, S. (2014). Effects of black pepper (*Piper nigrum*), turmeric powder (*Curcuma longa*) and coriander seeds (*Coriandrum sativum*) and their combinations as feed additives on growth performance, carcass traits, some blood parameters and humoral immune response of broiler chickens. *Asian-Australasian Journal of Animal Sciences, 27*(6), Article 847. https://doi.org/10.5713/ajas.2013.13644

Abou-Elkhair, R., Selim, S., & Hussein, E. (2018). Effect of supplementing layer hen diet with phytogenic feed additives on laying performance, egg quality, egg lipid peroxidation and blood biochemical constituents. *Animal Nutrition*, *4*(4), 394–400. https://doi.org/10.1016/j.aninu.2018.05.009

Agma Okur, A., & Unver Kayhan, E. (2019). Impacts of red pepper supplemented diets and different storage conditions on eggs obtained from free-range laying hens. *South African Journal of Animal Science*, *48*(5), Article 987. https://doi.org/10.4314/sajas.v48i5.19

Akter, Y., Kasim, A., Omar, H., & Sazili, A. Q. (2014). Effect of storage time and temperature on the quality characteristics of chicken eggs. *Journal of Food, Agriculture and Environment, 12*(3-4), 87-92.

Alam, M. A., Saleh, M., Mohsin, G. M., Nadirah, T. A., Aslani, F., Rahman, M. M., Roy, S. K., Juraimi, S. S., & Alam, M. Z. (2020). Evaluation of phenolics, capsaicinoids, antioxidant properties, and major macro-micro minerals of some hot and sweet peppers and ginger land-races of Malaysia. *Journal of Food Processing and Preservation, 44*(6), Article e14483. https://doi.org/10.1111/jfpp.14483

Al-matani, S. K., Al-Wahaibi, R. N. S., & Hossain, M. A. (2015). *In vitro* evaluation of the total phenolic and flavonoid contents and the antimicrobial and cytotoxicity activities of crude fruit extracts with different polarities from *Ficus sycomorus*. *Pacific Science Review A: Natural Science and Engineering, 17*(3), 103-108. https://doi.org/10.1016/j.psra.2016.02.002

Anas, M. A., Agus, A., Hanim, C., Babikian, H. J., & Jiaravanon, B. (2019). The effect of BAV addition as feed additive in laying hen ration on quality and chemical composition of egg. *IOP Conference Series: Earth and Environmental Science*, *387*(1), Article 012090. https://doi.org/10.1088/1755-1315/387/1/012090

Balenović, M., Savić, V., Janječić, Z., Popović, M., Šimpraga, B., Carović-Stanko, K., Bedeković, D., & Amšel Zelenika, T. (2018). Immunomodulatory and antimicrobial effects of selected herbs on laying hens. *Veterinarski Arhiv*, *88*(5), 673–686. https://doi.org/10.24099/vet.arhiv.0104

Boka, J., Mahdavi, A. H., Samie, A. H., & Jahanian, R. (2014). Effect of different levels of black cumin (*Nigella sativa* L.) on performance, intestinal Escherichia coli colonization and jejunal morphology in laying hens. *Journal of Animal Physiology and Animal Nutrition*, *98*(2), 373–383. https://doi.org/10.1111/jpn.12109

Cai, Y.-Z., Mei Sun, Jie Xing, Luo, Q., & Corke, H. (2006). Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sciences*, *78*(25), 2872–2888. https://doi.org/10.1016/j.lfs.2005.11.004

Campbell L., Raikos V., & Euston S. R. (2003) Modification of functional properties of egg-white proteins. *Molecular Nutrition and Food Research, 47*, 369–376. https://doi.org/10.1002/food.200390084

Cornescu, G. M., Vlaicu, A. P., Untea, A. E., Panaite, T. D., Oancea, A., & Saracila, M. (2022). The effects of diets incorporating natural source of tannins on laying hens' production performances and physical parameters of eggs. *Archiva Zootechnica*, *25*(2), 75–85. https://doi.org/10.2478/azibna-2022-0015

da Rosa, G., Dazuk, V., Alba, D. F., Galli, G. M., Molosse, V., Boiago, M. M., Souza, C. F., Abbad, L. B., Baldissera, M. D., Stefani, L. M., & Da Silva, A. S. (2020). Curcumin addition in diet of laying hens under cold stress has antioxidant and antimicrobial effects and improves bird health and egg quality. *Journal of Thermal Biology*, *91*. https://doi.org/10.1016/j.jtherbio.2020.102618

Eke, M. O., Olaitan, N. I., & Ochefu, J. H. (2013). Effect of storage conditions on the quality attributes of shell (table) eggs. *Nigerian Food Journal*, *31*(2), 18–24. https://doi.org/10.1016/S0189-7241(15)30072-2

Englmaierová, M., Tůmová, E., Charvátová, V., & Skřivan, M. (2014). Effects of laying hens housing system on laying performance, egg quality characteristics, and egg microbial contamination. *Czech Journal of Animal Science*, *59*(8), 345–352. https://doi.org/10.17221/7585-CJAS

Faber, M., Malan, L., Kruger, H. S., Asare, H., Visser, M., Mukwevho, T., Ricci, C., & Smuts, C. M. (2022). Potential of egg as complementary food to improve nutrient intake and dietary diversity. *Nutrients*, *14*(16), Article 3396. https://doi.org/10.3390/nu14163396

Feddern, V., Prá, M. C. De, Mores, R., Nicoloso, R. da S., Coldebella, A., & Abreu, P. G. de. (2017). Egg quality assessment at different storage conditions, seasons and laying hen strains. *Ciência e Agrotecnologia*, *41*(3), 322–333. https://doi.org/10.1590/1413-70542017413002317

Guimarães, R. R., de Oliveira, M. A., Oliveira, H. C., Doró, S. C. O. L., Machado, L. A., & de Oliveira, M. C. (2021). Turmeric powder in the diet of Japanese quails improves the quality of stored eggs. *Revista Brasileira de Saúde e Produção Animal, 22*, Article e2122052021. https://doi.org/10.1590/S1519-99402122052021

Hammershøj, M., Kidmose, U., & Steenfeldt, S. (2010). Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (*Daucus carota*) varieties as forage material for egg-laying hens. *Journal of the Science of Food and Agriculture, 90*(7), 1163-1171. https://doi.org/10.1002/jsfa.3937

Hanif, M. F., Ariyadi, B., Muhlisin, & Agus, A. (2023). Effect of dietary turmeric powder on egg quality and yolk cholesterol level of laying hens: a meta-analysis. *Livestock Research for Rural Development*, *35*(12), Article 114. https://www.lrrd.org/lrrd35/12/35114alia.html

Hanif, M. F., Ariyadi, B., Muhlisin, & Agus, A. (2024). Effect of pepper (*Capsicum* sp) on productivity and egg quality of laying hens: a meta-analysis. *Veterinary Integrative Sciences*, *22*(3), 749–767. https://doi.org/10.12982/VIS.2024.050

Haryatmi, D., & Susilowati, I. T. (2022). Determining the tannin content in Pisang Ambon (*Musa paradisiaca* L.) with the potential as anthelmintic. *Jurnal Kimia Riset, 7*(2), 118-124. https://doi.org/10.20473/jkr.v7i2.28859.

Haugh, R. (1937). The Haugh unit for measuring egg quality. *United States Egg and Poultry Magazine*, *43*, 522–555. https://sid.ir/paper/543065/en

Hernández-Pérez, T., Gómez-García, M. del R., Valverde, M. E., & Paredes-López, O. (2020). Capsicum annuum (hot pepper): An ancient Latin-American crop with outstanding bioactive compounds and nutraceutical potential. A review. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 2972–2993. https://doi.org/10.1111/1541-4337.12634

Hester, P. Y. (2017). Effects of Temperature and Storage Conditions on Eggs. In P. Y. Hester (Ed), *Egg Innovations and Strategies for Improvements* (pp. 125–134). Academic Press. https://doi.org/10.1016/B978-0-12-800879-9.00012-3

Hosseini, H., Esmaeili, N., Sepehr, A., Zare, M., Rombenso, A., Badierah, R., & Redwan, E. M. (2023). Does supplementing laying hen diets with a herb mixture mitigate the negative impacts of excessive inclusion of extruded flaxseed? *Animal Bioscience*, *36*(4), 629–641. https://doi.org/10.5713/ab.22.0183

Jin, Y. H., Lee, K. T., Lee, W. I., & Han, Y. K. (2011). Effects of storage temperature and time on the quality of eggs from laying hens at peak production. *Asian-Australasian Journal of Animal Sciences, 24*(2), 279-284. https://doi.org/10.5713/ajas.2011.10210

Kljak, K., Carović-Stanko, K., Kos, I., Janječić, Z., Kiš, G., Duvnjak, M., Safner, T., & Bedeković, D. (2021a). Plant carotenoids as pigment sources in laying hen diets: Effect on yolk color, carotenoid content, oxidative stability and sensory properties of eggs. *Foods*, *10*(4), Article 721 https://doi.org/10.3390/foods10040721

Kljak, K., Duvnjak, M., Bedeković, D., Kiš, G., Janječić, Z., & Grbeša, D. (2021b). Commercial Corn Hybrids as a Single Source of Dietary Carotenoids: Effect on Egg Yolk Carotenoid Profile and Pigmentation. *Sustainability*, *13*(21), Article 12287. https://doi.org/10.3390/su132112287

Li, X. L., He, W. L., Wang, Z. B., & Xu, T. S. (2016). Effects of Chinese herbal mixture on performance, egg quality and blood biochemical parameters of laying hens. *Journal of Animal Physiology and Animal Nutrition*, *100*(6), 1041–1049. https://doi.org/10.1111/jpn.12473

Madrigal-Portilla, J., Salas-Durán, C., & Macaya-Quirós, S. (2023). Efecto de temperatura y tiempo de almacenamiento sobre la calidad del huevo de gallinas. *Agronomía Mesoamericana*, *34*(2), Article 51223. https://doi.org/10.15517/am.v34i2.51223

Marina, Z., & Noriham, A. (2014). Quantification of total phenolic compound and in vitro antioxidant potential of fruit peel extracts. *International Food Research Journal, 21*(5), 1925-1929.

Morrine, A. O., Zen-Zi, W., Weih, G. B., Grant, A. H., Kamal, D., & David, J. B. (2018). Comparative analysis of capsaicin in twenty nine varieties of unexplored Capsicum and its antimicrobial activity against bacterial and fungal pathogens. *Journal of Medicinal Plants Research*, *12*(29), 544–556. https://doi.org/10.5897/jmpr2018.6667

Nagy, M., Socaci, S. A., Tofană, M., Pop, C., Mureşan, C., Pop Cuceu, A. V., Salanţă, L., & Rotar, A. M. (2015). Determination of Total Phenolics, Antioxidant Capacity and Antimicrobial Activity of Selected Aromatic Spices. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology*, *72*(1), 82-85 https://doi.org/10.15835/buasvmcn-fst:11094

Omana, D. A., Liang, Y., Kav, N. N. V., & Wu, J. (2011). Proteomic analysis of egg white proteins during storage. *Proteomics*, *11*(1), 144–153. https://doi.org/10.1002/pmic.201000168

Quan, T. H., & Benjakul, S. (2018). Quality, protease inhibitor and gelling property of duck egg albumen as affected by storage conditions. *Journal of Food Science and Technology, 55*(2), 513–522. https://doi.org/10.1007/s13197-017-2960-6.

Qiu, N., Ma, M., Zhao, L., Liu, W., Li, Y., & Mine, Y. (2012). Comparative proteomic analysis of egg white proteins under various storage temperatures. *Journal of Agricultural and Food Chemistry, 60*(31), 7746-7753. https://doi.org/10.1021/jf302100m

Réhault-Godbert, S., Guyot, N., & Nys, Y. (2019). The Golden Egg: Nutritional Value, Bioactivities, and Emerging Benefits for Human Health. *Nutrients*, *11*(3), Article 684. https://doi.org/10.3390/nu11030684

Romero-Luna, H. E., Colina, J., Guzmán-Rodríguez, L., Sierra-Carmona, C. G., Farías-Campomanes, Á. M., García-Pinilla, S., González-Tijera, M. M., Malagón-Alvira, K. O., & Peredo-Lovillo, A. (2023). Capsicum fruits as functional ingredients with antimicrobial activity: an emphasis on mechanisms of action. *Journal of Food Science and Technology*, *60*(11), 2725–2735. https://doi.org/10.1007/s13197-022-05578-y

Saki, A. A., Aliarabi, H., Hosseini Siyar, S. A., Salari, J., & Hashemi, M. (2014). Effect of a phytogenic feed additive on performance, ovarian morphology, serum lipid parameters and egg sensory quality in laying hen. *Veterinary Research Forum: An International Quarterly Journal*, *5*(4), 287–293. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4299994/

Saleh, A. A., Gawish, E., Mahmoud, S. F., Amber, K., Awad, W., Alzawqari, M. H., Shukry, M., & Abdel-Moneim, A. M. E. (2021). Effect of natural and chemical colorant supplementation on performance, egg-quality characteristics, yolk fatty-acid profile, and blood constituents in laying hens. *Sustainability (Switzerland)*, *13*(8), Article 4503. https://doi.org/10.3390/su13084503

Santana Santos, T., da Costa Lopes, C., Murilo Oliveira Junior, G., Moreira Santos, L., Santos Santana, C. C., & Melo Souza, D. (2019). The use of cinnamon powder in the diet of Japanese laying quail. *Acta Scientiarum. Animal Sciences*, *41,* Article e42963. https://doi.org/10.4025/actascianimsci.v41i1.42963

Slameto, Novianti, L., Fariroh, I., Rusdiana, R. Y., & Hariyono, K. (2021). Effects of potassium fertilizer on growth, capsaicin, and ascorbic acid content of local and hybrid chili (*Capsicum annum* L.). *Plant Cell Biotechnology and Molecular Biology, 22*(71-72), 337-345. https://ikprress.org/index.php/PCBMB/article/view/7302

Sünder, A., Wilkens, M., Böhm, V., & Liebert, F. (2022). Egg yolk colour in organic production as affected by feeding – Consequences for farmers and consumers. *Food Chemistry*, *382,* Article 131854. https://doi.org/10.1016/j.foodchem.2021.131854

Tabasum, S., Khare, S., & Jain, K. (2016). Spectrophotometric quantification of total phenolic, flavonoid, and alkaloid contents of *Abrus precatorius* L. seeds. *Asian Journal of Pharmaceutical and Clinical Research, 9*(2), 371-374. https://journals.innovareacademics.in/index.php/ajpcr/article/view/11018

Thohari, I., Umam Al Awwaly, K., Winirsya Apriliyani, M., & Aprillian, A. (2022). Effects of addition of TiO2 edible coatings and storage periods on the chicken eggs quality. *Asian Food Science Journal*, *21*(2), 14–21. https://doi.org/10.9734/afsj/2022/v21i230404

Tomczyk, Ł., Stępień, Ł., Urbaniak, M., Szablewski, T., Cegielska-Radziejewska, R., & Stuper-Szablewska, K. (2018). Characterisation of the mycobiota on the shell surface of table eggs acquired from different egg-laying hen breeding systems. *Toxins*, *10*(7), Article 293. https://doi.org/10.3390/toxins10070293

Tomczyk, Ł., Szablewski, T., Stuper-Szablewska, K., Nowaczewski, S., & Cegielska-Radziejewska, R. (2019). The influence of the conditions of acquisition and storage of table eggs on changes in their quality and the presence of mycobiota and Fusarium mycotoxins. *Poultry Science*, *98*(7), 2964–2971. https://doi.org/10.3382/ps/pez156

Vador, N., Vador, B., & Hole, R. (2012). Simple spectrophotometric methods for standardizing ayurvedic formulation. *Indian Journal of Pharmaceutical Sciences*, *74*(2), 161-163. https://doi.org/10.4103/0250-474X.103852

Valková, V., Ďúranová, H., Ivanišová, E., Galovičová, L., Godočíková, L., Borotová, P., Kunová, S., Miklášová, K., Lopašovský, Ľ., Mňahončáková, E., & Kačániová, M. (2021). Antioxidant and antimicrobial activities of fruit extracts from different fresh chili peppers. *Acta Scientiarum Polonorum Technologia Alimentaria*, *20*(4), 465–472. https://doi.org/10.17306/J.AFS.2021.0977

Walker, S., & Baum, J. I. (2022). Eggs as an affordable source of nutrients for adults and children living in food-insecure environments. *Nutrition Reviews*, *80*(2), 178–186. https://doi.org/10.1093/nutrit/nuab019

Wibowo, C. H., & Sudjatinah. (2023). The Effect of Different in Liquid Egg Yolk Storage Temperature on Physical, Chemical, Microbiological and Functional Properties. *IOP Conference Series: Earth and Environmental Science*, *1177*(1), Article 012045. https://doi.org/10.1088/1755-1315/1177/1/012045

Xia, B., Liu, Y., Sun, D., Liu, J., Zhu, Y., & Lu, L. (2018). Effects of green tea powder supplementation on egg production and egg quality in laying hens. *Journal of Applied Animal Research, 46*(1), 927-931. https://doi.org/10.1080/09712119.2018.1431240

Yuceer, M., & Caner, C. (2014). Antimicrobial lysozyme–chitosan coatings affect functional properties and shelf life of chicken eggs during storage. *Journal of the Science of Food and Agriculture*, *94*(1), 153–162. https://doi.org/10.1002/jsfa.6322