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Improvement in the Coloration and Quality of Japanese Quail Eggs Through Supplementation with Natural Pigments

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Abstract: This study evaluated the effects of natural pigments, such as paprika extract and marigold flower extract, on performance, egg quality, carotenoid content in the yolk, and shelf life of Japanese quail eggs. The experiment included 160 birds, divided into 4 treatment groups, as follows: 0.8% paprika extract, 0.8% marigold flower extract, 0.8% paprika/marigold (P/M) mixture, and a control group. The experiment lasted 84 days. The inclusion of paprika, marigold, or their combination did not affect performance parameters, except for feed conversion, which was significantly poorer in the supplemented groups compared to the control. An improvement in yolk color was observed, particularly in the marigold group, which showed higher concentrations of β-carotene (40 g/kg). Yolk color intensity, measured using the L*, a*, and b* scales, was enhanced in all supplemented groups. During storage, yolk weight increased after 5.72 days, while albumen weight showed a significant reduction starting at 4.30 days, with a more pronounced decrease after 6.6 days. Albumen diameter expanded after 15.7 days of storage, and the Haugh unit was significantly affected from the 17th day onward. Yolk percentage decreased after 4.06 days, while albumen and eggshell percentages decreased after 4.10 and 3.41 days, respectively. These results suggest that paprika and marigold extracts are effective in improving yolk color and maintaining egg quality without compromising performance. However, a poorer feed conversion by mass was observed in the groups fed with the paprika, marigold, and the (P/M) mixture, compared to the control group. These natural pigments also positively impacted egg quality, particularly in terms of color intensity and internal parameters, and helped maintain egg quality during storage, meeting consumer expectations for high-quality products. Future studies should focus on evaluating the antioxidant effects of these pigments in eggs, as this could provide a deeper understanding of their potential benefits, both in terms of nutritional quality and shelf-life enhancement.

Keywords: beta-carotene; egg quality; marigold flower; paprika extract; shelf life

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1. Introduction

The quail farming industry in Brazil has been intensifying, emerging as an activity of growing economic interest [1,2]. The production of high-quality eggs is essential to meet the increasing consumer demand for quail eggs. In Brazil, where quail farming is predominantly focused on egg production, the industry is rapidly integrating with the commercial poultry sector, driven by the development of new production technologies [3].

The appearance of eggs, particularly yolk color, plays a significant role in consumers' purchasing decisions, making it common to include pigments in poultry feed. This becomes especially important when alternative ingredients to corn, such as sorghum, are used, as they have a lower concentration of carotenoids, resulting in less pigmented yolks [4,5]. Most consumers prefer eggs with more orange-colored yolks, prompting the industry to use pigments in bird diets [6].

Although synthetic pigments have been widely used in poultry feed, their potential health risks and the growing consumer demand for more natural products make plant-based alternatives an increasingly attractive option. Natural pigments, such as paprika extract and marigold flowers, not only offer a safe solution but also contribute to improved egg quality, offering a more sustainable and consumer-friendly alternative. The use of natural pigments in quail feed has gained attention due to their potential to enhance yolk color, which is a key quality parameter for consumer acceptance [7,8].

Paprika (*Capsicum annuum*), obtained by grinding bell pepper (*Capsicum annuum* L.), is a good option as a natural pigment, containing 4 to 8 g/kg of xanthophylls, with 50 to 70% capsanthin, a red–orange pigment [9]. Marigold (*Calendula officinalis*), obtained from the dried flowers of the plant, is also a viable alternative as a natural pigment, containing 8 to 15 g/kg of xanthophylls, predominantly lutein and zeaxanthin, responsible for the yellow–orange color [10]. These pigments have not only visual effects but may also improve other important sensory properties of eggs, such as taste, texture, and odor, which are essential for consumer acceptance.

It is important to consider that phytogenic additives do not only present the desired effects but may also influence the digestive system and immunity of animals [11]. These effects on overall health could indirectly influence egg production and quality, contributing to higher performance and better egg characteristics.

Furthermore, the main expected effect of these pigments, beyond intensifying the orange–red tone, is the antioxidant effect due to their high lutein content. Studies suggest that antioxidants, such as lutein and zeaxanthin, may significantly contribute to increasing the shelf life of eggs by slowing down lipid oxidation and preserving the nutritional quality of yolks for longer periods [12]. Additionally, the use of natural pigments can reduce the degradation of sensory compounds in the eggs, ensuring that the eggs retain their freshness and quality over time.

The primary hypothesis of this research is that the inclusion of natural pigments in the diet of Japanese quail will positively influence not only yolk color but also egg quality parameters, such as nutritional content, sensory properties, and shelf life. Specifically, this study aims to evaluate the impact of plant-based extracts on performance variables, carotenoid content in the yolk, and the overall quality of eggs during different storage periods.

2. Materials and Methods

The experiment was carried out at the poultry and quail facility of the Department of Animal Science at the Federal University of Grande Dourados. The study was submitted to and approved by the Animal Ethics Committee of the Federal University of Grande Dourados (UFGD) under protocol number 16/2020, with the approval date being the 19 August 2022, in Dourados-MS, Brazil.

The Japanese quails (*Coturnix coturnix japonica*) used in this study were obtained from Granja Fujikura, located in Suzano, São Paulo, Brazil. The birds were commercially sourced and had been previously housed under standard commercial rearing conditions. From 1 to 21 days of age, they were raised in brooding circles under controlled environmental conditions. During this period, the temperature was maintained according to the recommended values for each phase, with an average of 35 °C in the first week, 30 °C in the second week, and 28 °C in the third week, with gradual reductions until reaching room temperature.

Throughout this phase, the birds were fed a basal diet formulated according to the nutritional recommendations of [13]. Water and feed were provided ad libitum, with water supplied through pendulum drinkers and pressure cup drinkers, and feed distributed in tube feeders, adjusted according to the birds' developmental phase.

At 21 days of age, the quails were transferred to battery cages under standard housing conditions, where they remained until they reached peak egg production. When the birds reached a 90% laying rate, at approximately 50 days of age, the experiment was initiated and lasted 84 days, divided into three 28-day periods. The birds were housed in a masonry poultry house measuring 6.0 m in length, 2.5 m in width, and 3.5 m in ceiling height, with a concrete floor and a fiber cement roof. The facility featured 0.60 m high sidewalls and a 0.50 m long eave, as well as yellow polyethylene external curtains with manual adjustment. Additionally, two climate control systems were installed to regulate the poultry house temperature.

During the initial phase (1 to 21 days of age), the lighting program consisted of 24 h of artificial light, using the same lamps that served as a heat source, until the birds reached 21 days of age. After being transferred to the cages, from 21 to 42 days of age, a natural photoperiod was used to prevent premature sexual maturity.

At the onset of the laying phase, a 16 h daily light regimen was implemented and maintained throughout the entire experimental period. This lighting schedule was controlled by an automatic timer, which regulated the switching on and off of the lights during the night and early morning, following standard procedures used in commercial farms.

A completely randomized design was used, with 8 replications, 4 treatments, and 5 quails per experimental unit at peak laying time, totaling 160 birds. The birds were housed in cages measuring 50 × 50 × 16.5 cm (length × width × height), with two compartments of 25 × 50 cm, totaling 1250 cm^2. The animal density per experimental unit was 250 cm^2/bird.

The experimental feeds were provided ad libitum three times a day in galvanized metal trough feeders running throughout the entire length of the cages. The feeders were divided according to each treatment and replication.

Water was also provided ad libitum via nipple drinkers. The treatments were as follows: T1, control treatment; T2, 0.8% paprika extract; T3, 0.8% 659; and T4, 0.4% paprika extract and 0.4% marigold. The basal diet in this experiment was formulated with corn and soybean meal, in accordance with the feed composition and nutritional requirements outlined by [13], as shown in Table 1.

Daily management during the early laying phase included tasks, such as collecting and documenting egg production, which encompassed broken, cracked, soft-shelled, and shell-less eggs. Additionally, feed was provided, egg collection trays were cleaned, and the temperature (both maximum and minimum) and relative humidity (RH) were monitored. Temperature and RH were recorded daily at 8:00 AM using maximum–minimum thermometers, along with dry- and wet-bulb thermometers, placed at the center of the poultry house at the height of the birds' backs. The recorded environmental conditions showed a minimum temperature of 18.31 ± 0.33 °C, a maximum temperature of 29.6 ± 0.17 °C, a maximum RH of 83.0 ± 2.5%, and a minimum RH of 46.0 ± 1.5%. Climate control systems and curtain adjustments were made based on these daily temperature readings to ensure that the birds experienced optimal environmental conditions.

Table 1. Percentages and calculated compositions of the experimental diets during the production phase.

	Natural Pigments			
	Control	**Paprika**	**Marigold**	**(P/M) Mixture**
Ingredients	**0%**	**0.8%**	**0.8%**	**0.8%**
Corn	56.291	56.291	56.291	56.291
Soybean meal 45%	30.707	30.707	30.707	30.707
Inert	1.069	0.989	0.989	0.989
Paprika	0.0	0.08	0.0	0.04
Marigold	0.0	0.0	0.08	0.04
Soybean oil	2.674	2.674	2.674	2.674
Limestone	6.95	6.95	6.95	6.95
Dicalcium phosphate	1.026	1.026	1.026	1.026
Salt	0.343	0.343	0.343	0.343
DL-methionine 98%	0.417	0.417	0.417	0.417
L-lysine HCL 76%	0.323	0.323	0.323	0.323
[1] Vitamin premix	0.1	0.1	0.1	0.1
[2] Mineral premix	0.1	0.1	0.1	0.1
Nutritional Composition Calculated				
ME (Kcal/Kg)	2800.00	2800.00	2800.00	2800.00
Crude protein (%)	18.920	18.920	18.920	18.920
Digestible lysine (%)	1.1490	1.1490	1.1490	1.1490
Methionine + Cyst (%)	0.9421	0.9421	0.9421	0.9421
Digestible tryptophan (%)	0.186	0.186	0.186	0.186
Digestible threonine (%)	0.733	0.733	0.733	0.733
Calcium (%)	2.990	2.990	2.990	2.990
Available phosphorus (%)	0.282	0.282	0.282	0.282
Sodium (%)	0.147	0.147	0.147	0.147

Inert: Inert in the feed refers to substances or ingredients that do not have direct nutritional value for the animals, meaning they do not contribute significantly to the supply of energy, proteins, vitamins, or minerals. [1] Vitamin premix/kg of diet: folic acid (Min.) 145.4 mg; pantothenic acid (Min.) 5931.6 mg; choline (Min.) 121.8 g; niacin (Min.) 12.9 g; selenium (Min.) 480.0 mg; vitamin A (Min.) 5000.0 IU; vitamin B12 (Min.) 6500.0 mcg; vitamin B2 (Min.) 2000.0 mg; vitamin B6 (Min.) 250.0 mg; vitamin D3 (Min.) 1,850,000.0 IU; vitamin E (Min.) 4500.0 IU; and vitamin K3 (Min.) 918.0 mg. [2] Mineral premix/kg: copper (Min.) 7000.0 mg; iron (Min.) 50.0 g; iodine (Min.) 1500.0 mg; manganese (Min.) 67.5 g; zinc (Min.) 45.6 g.

2.1. Performance

The performance variables were measured throughout the entire experimental period, which lasted 84 days. These assessments included bird/day feed conversion (g), egg production (%), marketable egg production (%), egg conversion per dozen (%), egg mass (kg), feed conversion by mass (kg/kg), and production viability (%). At the end of each experimental period, the leftover feed from each group was weighed and subtracted from the total amount provided to determine the actual feed intake. In cases of bird mortality during the period, the average feed consumption was adjusted to reflect the true average consumption per experimental unit. This adjustment was carried out by weighing the feed until the bird's death, dividing the total by the number of birds in the pen, and subtracting the average consumption of the deceased birds to correct the final value.

Egg production was calculated by counting the total number of eggs produced, including broken, cracked, and abnormal eggs (such as soft-shelled and shell-less eggs). This number was then expressed as a percentage based on the average number of birds during the period (eggs/bird/day) and the total number of birds initially housed at the start of the experiment (eggs/initially housed bird).

To calculate marketable egg production, the number of broken, cracked, soft-shelled, and shell-less eggs was subtracted from the total egg production for each 28-day period. The ratio between intact and total eggs produced during each period was then computed.

The weight of all intact eggs produced in each replication was recorded during the last 3 days of each 28-day period to calculate the average weight. The average egg weight was then multiplied by the egg production per bird per day to determine the total egg mass. Feed conversion per dozen eggs was calculated by dividing the total feed consumption in kilograms by the number of dozen eggs produced (kg/dozen). Feed conversion by egg mass was calculated by dividing the total feed intake (kg) by the total egg mass (kg/kg).

Bird mortality was monitored daily. At the end of the experimental period, the bird viability rate was calculated by subtracting the number of dead birds from the number of live birds, and the result was expressed as a percentage.

2.2. Carotenoid Content

The carotenoid content was determined according to the method described by [14]. Carotenoid extraction was performed via 2 g of sample from a pool of 3 eggs, which were previously macerated with cold acetone through successive extractions followed by filtration. The filtrate was transferred to a separation funnel, where petroleum ether was added, forming two phases, namely an upper phase containing petroleum ether with the carotenoids and a lower phase containing water and acetone.

The solution was then collected in a 50 mL volumetric flask and covered with aluminum foil to preserve the carotenoids. The extracts were analyzed using a Biochrom Libra S60PC spectrophotometer, manufactured by Biochrom, headquartered in Cambridge, UK, at 450 nm.

2.3. Egg Quality

To evaluate the external and internal quality of the eggs, three intact eggs from each plot were collected during the last three days of each experimental period in the morning, totaling 864 eggs. Each egg was individually weighed using a semi-analytical scale, and the specific gravity was then measured. Specific gravity was determined by immersing the eggs in saline solutions with varying densities, ranging from 1.065 to 1.125, increasing by 0.005 for each solution, following the methodology outlined by [2]. The densities were adjusted with a hydrometer, and the eggs were submerged in the solutions starting from the lowest to the highest salinity concentrations.

Next, the eggs were cracked open, and the albumen, yolk, and shell were manually separated. The yolks were individually weighed using a precision scale. The shells were washed with running water and naturally dried for 72 h. After drying, the shells were left at room temperature and then weighed individually.

The albumen weight was calculated by subtracting the yolk and shell weights from the total egg weight. The heights of the yolk and albumen, as well as the yolk's diameter, were measured using a digital caliper and tripod, with the yolk height measured at the center and the albumen height measured approximately 0.5 cm from the yolk. The yolk index was calculated as the ratio between the yolk height and its diameter. The Haugh unit was calculated using a mathematical model based on the methodology of [15].

Yolk color was assessed using a portable colorimeter (Minolta CR 410 model), manufactured by Minolta, headquartered in Osaka, Japan, which measured luminosity (L*), red color (a*), and yellow color (b*) at three different points on the yolk surface. Additionally, eggshell color was evaluated using a La Roche color fan.

2.4. Egg Storage

To assess the effect of time on egg coloration, a completely randomized design with a 4 × 3 factorial arrangement was used, with four diets and three storage periods and eight replications. Three eggs from each replication were considered one experimental unit. Three storage periods were evaluated, namely, 0 (fresh egg), 7, and 14 days of storage, with the experimental diets, resulting in 288 eggs being analyzed.

The eggs were stored (7 and 14 days) in a room with no direct sunlight which was dry and well-ventilated. The minimum and maximum temperatures were 32.8 ± 0.2 °C and 21.9 ± 0.15 °C, respectively, whereas the maximum and minimum relative humidities were 69 ± 1.3% and 41.5 ± 1.5%, respectively. The analyses were performed as described in the egg quality section.

2.5. Statistical Analysis

The data were analyzed using the Statistical Analysis System (SAS) software package, version 9.4 [16]. The normality of the residuals was assessed using the Shapiro–Wilk test, while homogeneity of variances was checked with Levene's test. To evaluate the main effects of pigment sources on performance, egg quality, and carotenoids, means were compared using Tukey's test. For analyses involving storage time, analysis of variance (ANOVA) was performed using the PROC MIXED procedure to examine the interaction between pigment factors and storage time, as well as the individual effects of each factor. To explore the interactions, the storage time sum of squares was decomposed using orthogonal polynomials, and the corresponding regression equations were adjusted. A significance level of 5% was used for all statistical analyses.

3. Results

Considering performance, the inclusion of paprika extract, marigold, or their combination in the diet of laying Japanese quails did not result in significant differences ($p > 0.05$) between treatments, except for feed conversion (Table 2). Feed conversion by mass (kg/kg) showed a significant difference ($p = 0.013$) between treatments, with the groups receiving paprika, marigold, and the (P/M) mixture showing poorer feed conversion compared to the control group.

Table 2. Performance and β-carotene content in the yolk of quail eggs fed different sources of natural pigments.

Variables	Natural Pigment Sources				SEM	*p*-Value
	Control	Paprika	Marigold	(P/M) Mixture		
Feed intake g/hen/day	25.85	25.51	25.79	25.74	0.479	0.96
Egg production (%)	95.07	92.38	92.5	94.22	1.275	0.35
Commercial egg production (%)	96.73	95.04	95.4	96.29	1.173	0.71
Feed conversion by dozen	3.32	3.16	3.19	3.12	0.260	0.19
Egg mass (g)	9.25	9.49	9.41	9.5	0.171	0.150
Feed conversion by mass (kg/kg)	2.48 [b]	2.69 [a]	2.75 [a]	2.71 [a]	0.065	0.013

SEM: Standard error of the mean. Means followed by different letters in the same row are significantly different at the 5% probability level according to the Tukey test.

The carotenoid content in the yolk varied significantly ($p < 0.05$) among the different pigment supplement sources. The highest concentration of β-carotene in the yolk was detected in birds fed diets containing marigold extract, which was significantly greater than that in those fed with paprika or the (P/M) mixture, which presented similar values. Compared with the control, all the pigment sources resulted in a significantly ($p < 0.05$) greater

carotenoid content, which was associated with the lowest concentration of β-carotene in the yolk (Figure 1).

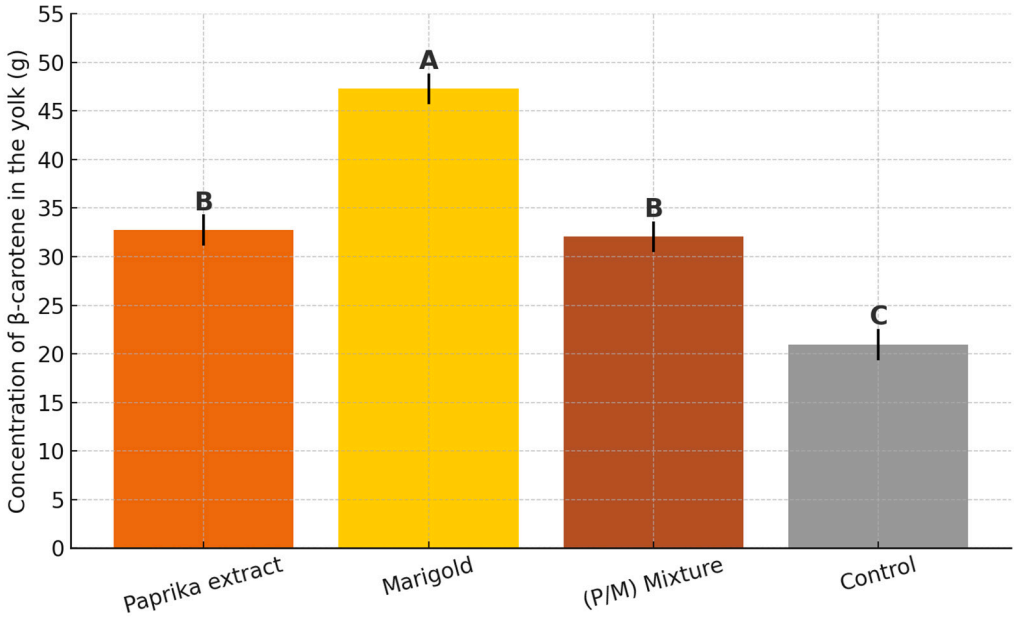


Figure 1. β-carotene concentration (g per g of egg yolk) in the egg yolk of birds fed different natural pigment sources. The uppercase letters indicate statistically significant differences ($p < 0.05$) between treatment groups. Treatments sharing the same letter are not significantly different from each other, while those with different letters represent statistically distinct means based on post hoc comparison tests (e.g., Tukey's test).

The inclusion of paprika extract, marigold, or their combination in the diet of laying Japanese quail had a significant effect ($p < 0.05$) on egg quality variables, such as egg weight, shell weight, albumen weight, Haugh unit, yolk percentage, and colorimetry (L. a*. and b*), Table 3.

The inclusion of marigold, paprika, or their combination in the diet of laying Japanese quail resulted in significant changes in various egg quality parameters compared to the control group. Specifically, egg weight was notably lower in the marigold, paprika, and (P/M) mixture ($p < 0.0001$), as was shell weight ($p < 0.0001$). Similarly, albumen weight decreased significantly in these groups ($p < 0.0001$). On the other hand, the Haugh unit, a key indicator of egg quality, was significantly higher in the marigold group compared to the control ($p = 0.0142$) (Table 3). Furthermore, yolk percentage increased significantly in the marigold group, with the paprika and (P/M) mixture showing intermediate results ($p = 0.0306$). Finally, significant changes in colorimetry were observed, with the 'a*' and 'b*' values indicating a stronger color intensity in the paprika and (P/M) mixture, as compared to the control ($p < 0.0001$ for 'a*' and $p = 0.0005$ for 'b*') (Table 3).

The inclusion of marigold, paprika, or their combination (P/M) in the diet of laying Japanese quail resulted in significant changes in various egg quality parameters compared to the control group. Specifically, egg weight was notably lower in the marigold, paprika, and (P/M) mixture treatments ($p < 0.0001$), as was shell weight ($p < 0.0001$). Similarly, albumen weight decreased significantly in these groups ($p < 0.0001$).

On the other hand, the Haugh unit, a key indicator of egg quality, was significantly higher in the marigold group compared to the control ($p = 0.015$) (Table 4). Furthermore, yolk percentage increased significantly in the marigold group, with the paprika and (P/M) mixture showing intermediate results ($p = 0.0306$). Significant changes in colorimetry were observed, with the 'a*' and 'b*' values indicating a stronger color intensity in the paprika

and (P/M) mixture, compared to the control ($p < 0.0001$ for 'a*' and $p = 0.0005$ for 'b*') (Table 4).

Table 3. Egg quality of fresh eggs with the inclusion of different pigment sources in the diet.

Variables	Pigment Additive				SEM	*p*-Value
	Control	Paprika	Marigold	(P/M) Mixture		
Egg weight (g)	11.215 a	10.259 b	10.164 b	10.091 b	0.079	<0.0001
Yolk weight (g)	3.637	3.62	3.68	3.554	0.034	0.6219
Shell weight (g)	0.904 a	0.792 b	0.788 b	0.779 b	0.01	<0.0001
Albumen weight (g)	6.642 a	5.789 b	5.695	5.757 b	0.085	<0.0001
Specific gravity	1.072	1.066	1.066	1.078	0.003	0.4373
Color fan	4.687 c	10.791 a	8.978 b	10.875 a	0.223	<0.0001
Albumen height (mm)	4.196	4.433	4.558	4.435	0.06	0.1951
Yolk height (mm)	10.56	10.474	10.426	10.309	0.057	0.4843
Yolk diameter (mm)	23.609	24.326	23.877	23.8	0.117	0.1735
Haugh unit	87.863 b	89.957 ab	90.829 a	90.105 ab	0.342	0.0142
Yolk index	0.449	0.442	0.483	0.426	0.003	0.0959
% yolk	32.952 c	36.355 b	36.683 a	35.337 ab	0.492	0.0306
% albumen	58.964	56.968	55.764	56.83	0.419	0.0527
% shell	8.083	7.832	7.897	7.829	0.121	0.8701
L	54.047 b	55.826 a	55.424 a	55.417 a	0.184	0.0033
a*	2.224 d	6.663 b	4.232 c	8.285 a	0.321	<0.0001
b*	35.441 c	37.201 b	42.119 a	38.882 ab	0.590	0.0005

SEM: Standard error of the mean. Means followed by different letters in the same row are significantly different at the 5% probability level according to the Tukey test.

Table 4. Quality of quail eggs with the inclusion of different sources of natural pigments during a storage period of 0, 7, and 14 days.

Storage		Pigment Additive			SEM	*p*-Value		
		0	7	14		Treat	Temp	T*T
Egg weight (g)	Control	11.21	10.45	10.47	0.040	<0.0001	0.8099	0.5482
	Marigold	10.16	10.27	10.47				
	(P/M) mixture	10.09	10.28	10.11				
	Paprika	10.25	10.48	10.50				
Yolk weight (g)	Control	3.66	3.60	3.82	0.023	0.1388	<0.0001	0.1586
	Marigold	3.68	3.37	4.01				
	(P/M) mixture	3.55	3.45	3.72				
	Paprika	3.70	3.49	3.86				
Albumen weight (g)	Control	6.64	5.89	5.73	0.042	0.0112	<0.0001	0.0006
	Marigold	5.69	6.11	5.58				
	(P/M) mixture	5.75	6.03	5.39				
	Paprika	5.78	6.11	5.61				
Shell weight (g)	Control	0.90	0.88	0.89	0.005	<0.0001	<0.0001	<0.0001
	Marigold	0.79	0.78	0.89				
	(P/M) mixture	0.78	0.79	0.90				
	Paprika	0.79	0.80	0.92				
Specific gravity	Control	1.07	1.07	1.07	0.002	0.1453	0.2074	0.7186
	Marigold	1.07	1.07	1.07				
	(P/M) mixture	1.08	1.08	1.07				
	Paprika	1.07	1.07	1.07				

Table 4. *Cont.*

Storage		Pigment Additive			SEM	*p*-Value		
		0	7	14		Treat	Temp	T*T
% yolk	Control	32.95	34.97	47.09	0.622	0.939	<0.0001	0.2326
	Marigold	36.33	33.22	46.07				
	(P/M) mixture	35.33	33.71	45.55				
	Paprika	35.21	33.72	45.51				
% albumen	Control	58.96	57.10	45.33	0.597	0.966	<0.0001	0.3062
	Marigold	55.76	59.08	45.21				
	(P/M) mixture	56.83	58.53	45.83				
	Paprika	56.96	58.50	45.65				
% shell	Control	8.08	8.46	8.64	0.095	0.0939	<0.0001	0.0649
	Marigold	7.79	7.68	8.71				
	(P/M) mixture	7.89	7.74	8.88				
	Paprika	7.82	7.76	8.92				
Shell thickness (mm)	Control	0.21	0.20	0.20	0.002	0.117	0.9053	0.1799
	Marigold	0.21	0.21	0.21				
	(P/M) mixture	0.20	0.21	0.21				
	Paprika	0.21	0.22	0.21				
Albumen height (mm)	Control	4.19	3.13	2.57	0.061	0.0603	<0.0001	0.4917
	Marigold	4.55	3.12	2.67				
	(P/M) mixture	4.43	3.10	2.68				
	Paprika	4.43	3.40	2.77				
Yolk height (mm)	Control	10.56	8.54	7.02	0.690	<0.0001	<0.0001	<0.0001
	Marigold	10.42	7.66	5.66				
	(P/M) mixture	10.47	7.70	5.79				
	Paprika	10.30	7.96	5.63				
Yolk diameter (mm)	Control	23.60	26.39	30.55	0.178	<0.0001	<0.0001	0.0006
	Marigold	23.87	27.92	33.50				
	(P/M) mixture	23.80	27.62	33.03				
	Paprika	24.32	27.77	33.46				
Haugh unit	Control	87.86	82.19	78.34	0.392	0.015	<0.0001	0.2949
	Marigold	90.82	82.22	79.07				
	(P/M) mixture	90.11	82.07	79.31				
	Paprika	89.95	83.89	79.80				
Yolk index	Control	0.45	0.33	0.22	0.003	<0.0001	<0.0001	0.0006
	Marigold	0.44	0.28	0.17				
	(P/M) mixture	0.44	0.28	0.18				
	Paprika	0.42	0.29	0.17				
L	Control	55.82	65.14	61.28	0.280	<0.0001	<0.0001	<0.0001
	Marigold	55.42	57.57	55.94				
	(P/M) mixture	54.04	55.56	56.51				
	Paprika	55.41	56.29	56.79				
a	Control	−2.22	−2.84	−2.77	0.212	<0.0001	<0.0001	<0.0001
	Marigold	2.22	3.54	8.11				
	(P/M) mixture	8.28	8.17	7.31				
	Paprika	6.66	7.00	6.74				
b	Control	35.44	44.99	43.89	0.513	<0.0001	<0.0001	<0.0001
	Marigold	42.11	49.50	49.70				
	(P/M) mixture	38.88	45.36	49.30				
	Paprika	37.20	43.26	50.84				

SEM: Standard error of the mean. T*T: Treatment versus time. Treat: treatment. Temp: temperature.

There was a significant interaction between different pigment sources and storage periods ($p < 0.05$) on various egg quality parameters (Table 5). The regression equations and statistical models provided a more detailed understanding of the behavior of these

variables over time. However, the analysis of egg weight and yolk weight did not reveal any significant interaction between pigment sources and storage time (Table 5). Only isolated effects of pigment sources on egg weight and storage time on yolk weight were observed. Nevertheless, significant interactions between storage time and pigment sources were identified for albumen weight, eggshell weight, yolk height, yolk diameter, yolk index, and color parameters (L, a, and b).

Table 5. Regression equations for the quality of quail eggs with the inclusion of different sources of natural pigments during a storage period of 0, 7, and 14 days.

Variable	Effect	*p*-Value	Equation	R^2
Egg yolk weight (g)	Quad	0.0004	$y = 0.00555x^2 - 0.06343x + 3.65231$	0.1713
Shell weight (marigold)	Quad	0.0027	$y = 0.00123x^2 - 0.01060x + 0.796689$	0.1627
Shell weight (P/M) mixture	Quad	0.0056	$y = 0.00106x^2 - 0.00660x + 0.78921$	0.2102
Shell weight (paprika)	Quad	0.0094	$y = 0.00109x^2 - 0.00549x + 0.79282$	0.2254
Albumen weight (control)	Lin	<0.0001	$y = -0.06493x + 6.54404$	0.1250
Albumen weight (marigold)	Quad	0.005	$y = -0.00974x^2 + 0.12849x + 5.69512$	0.0567
Albumen weight (P/M) mixture	Quad	0.0023	$y = -0.00944x^2 + 0.10598x + 5.75765$	0.0910
Albumen weight (paprika)	Quad	0.0374	$y = -0.000841x^2 + 0.10521x + 5.78906$	0.0344
Albumen height	Quad	<0.0001	$y = 0.00710x^2 - 0.22286x + 4.40604$	0.4864
Yolk height (control)	Lin	<0.0001	$y = -0.25257x + 10.47744$	0.7207
Yolk height (marigold)	Quad	0.0063	$y = 0.00766x^2 - 0.44751x + 10.42604$	0.8693
Yolk height (P/M) mixture	Quad	0.0018	$y = 0.00879x^2 - 45723x + 10.47396$	0.8656
Yolk height (paprika)	Lin	<0.0001	$y = -0.33420x + 10.30687$	0.8311
Yolk diameter (control)	Lin	<0.0001	$y = 0.49634x + 23.38139$	0.6179
Yolk diameter (marigold)	Quad	0.0464	$y = 0.01577x^2 + 0.46723x + 23.87729$	0.7710
Yolk diameter (P/M) mixture	Quad	0.0399	$y = 0.01621x^2 + 0.43286x + 23.800$	0.7577
Yolk diameter (paprika)	Quad	0.0016	$y = 0.02284x^2 + 0.33311x + 24.32646$	0.7901
Haugh unit	Quad	<0.0001	$y = 0.03704x^2 - 1.27246x + 89.68903$	0.4627
Index yolk (control)	Lin	<0.0001	$y = -0.01609x + 0.44612$	0.7889
Index yolk (marigold)	Quad	<0.0001	$y = 0.00056675x^2 - 0.02709x + 0.43831$	0.9085
Index yolk (P/M) mixture	Quad	<0.0001	$y = 0.00059524x^2 - 0.02729x + 0.44225$	0.8971
Index yolk (paprika)	Lin	<0.0001	$y = -0.01835x + 0.42280$	0.8723
% yolk	Quad	<0.0001	$y = 0.13457x^2 - 1.09161x + 34.95879$	0.3481
% albumen	Quad	<0.0001	$y = -0.14272x^2 + 1.16782x + 57.132$	0.4110
% shell	Quad	<0.0001	$y = 0.00889x^2 - 0.06068x + 7.90059$	0.1167
L (control)	Quad	<0.0001	$y = -0.13452x^2 + 2.27329x + 55.82667$	0.3500
L (marigold)	Quad	0.0003	$y = -0.03864x^2 + 0.57836x + 55.42437$	0.0960
L (P/M) mixture	Lin	<0.0001	$y = 0.17653x + 54.14128$	0.1697
L (paprika)	Lin	<0.0001	$y = 0.09851x + 55.48014$	0.0657
a (control)	Lin	<0.0001	$y = -0.03894x - 2.34003$	0.0365
a (marigold)	Quad	0.0003	$y = 0.03319x^2 - 0.04495x + 2.23271$	0.4743
b (control)	Quad	<0.0001	$y = -0.010878x^2 + 2.12662x + 35.444146$	0.4206
b (marigold)	Quad	0.0068	$y = -0.07334x^2 + 1.56921x + 42.11187$	0.1887
b (P/M) mixture	Lin	<0.0001	$y = -0.02585x^2 + 1.10641x + 38.88229$	0.3437
b (paprika)	Lin	<0.0001	$y = 0.01549x^2 + 0.75789x + 37.20188$	0.4745

Lin: Linear. Quad: Quadratic.

According to the regression equations in Table 5, yolk weight showed an increase after 5.72 days of storage, while albumen weight showed a significant reduction starting from 4.30 days, with a more pronounced decrease after 6.6 days. Albumen diameter expanded after 15.7 days of storage, whereas the Haugh unit was significantly affected from the 17th day onward. Yolk percentage showed a weight reduction after 4.06 days, while the percentages of albumen and eggshell decreased after 4.10 and 3.41 days, respectively.

The albumen weight demonstrated a linear effect, with gradual losses as storage time increased. Both yolk height and yolk index showed a gradual reduction, while yolk diameter progressively increased over time.

The interactions between storage time and treatments revealed distinct effects on egg characteristics. Eggshell weight continuously decreased with storage time, with a less pronounced reduction observed in the control group. Similarly, yolk height decreased over time, but this reduction was slower in the control group compared to the other treatments. Egg luminosity (L) followed the same trend, progressively decreasing over time. In contrast, redness intensity (a)* increased over the storage period, being more evident in the (P/M) mixture and paprika treatments. Similarly, yellowness intensity (b)* gradually increased over time, with the marigold treatment showing the most significant effect.

4. Discussion

The results of this study indicated that the inclusion of paprika extract, marigold, or their combination (P/M) in the diet of Japanese quails affected performance and egg quality in distinct ways. In terms of performance, no significant differences were observed in feed intake or egg production among the treatments. However, poorer feed conversion was observed in the groups fed paprika, marigold, and the (P/M) mixture, compared to the control group. Nevertheless, it is important to highlight that, while feed conversion was affected, this effect should not be considered directly negative, as differences in other egg quality parameters may offset this effect.

Regarding egg quality, the inclusion of natural pigments had a significant impact on various parameters, such as egg weight, shell weight, albumen weight, Haugh unit, yolk percentage, and colorimetry. The marigold treatment resulted in higher color intensity, with the paprika and (P/M) mixtures showing intermediate results. This effect is consistent with the observed data, which show a significant increase in color intensity in eggs supplemented with pigments.

The concentration of β-carotene in the yolk was significantly higher in the marigold-fed group, which supports the idea that natural pigments, such as carotenoids, are effective at improving yolk coloration. The interaction between treatments and storage time revealed significant changes in shell weight and albumen weight, as well as a gradual increase in color intensity over time, highlighting the effects of natural pigments not only on the immediate quality of eggs but also on their preservation during storage.

These findings contrast with the results of [17], who reported that the supplementation of laying hen diets with red pepper- and marigold-based pigments significantly influenced bird performance. Additionally, Ref. [18] found that a sorghum-based diet, which has a lower carotenoid concentration, resulted in lower feed consumption and consequently affected egg production and mass. In our study, supplementation with paprika and marigold did not show a significant negative impact on egg production but rather beneficial changes in egg quality, as evidenced by the observed results.

Pro-vitaminic or non-pro-vitaminic carotenoids are absorbed primarily in the intestine, where they are incorporated into chylomicrons and transported via the lymphatic system to the bloodstream. Their bioavailability depends on the amount included in the diet, transport, absorption, metabolism, and the animal's genetics [19]. These pigments are transported to lipid-rich tissues, such as the egg yolk, and their deposition is influenced by the amount of lipids in the diet [20]. According to [21], carotenoids that are not converted into vitamin A are absorbed in free form, associated with fatty acids, dissolved in micelles, and transported as lipoproteins in the blood.

Differences in the rate of pigment deposition in the yolk between stereoisomers should also be considered [22]. The 3R, 3′R carotenoids are predominant in plants and are de-

posited more efficiently, such as zeaxanthin [23]. On the other hand, trans-carotenoids are more effective than cis-carotenoids due to their reddish hue. The proportion of trans-carotenoids is higher in plants (60 to 90%) [23], but the levels of cis-carotenoids increase during processing [17]. The proportion of the cis isomer in the yolk is higher due to biotransformation in hens [22]. Natural xanthophylls from *Tagetes erectus* and *Capsicum* sp. demonstrated efficacy comparable to synthetic pigments in egg yolk coloration, with intensity dependent on the concentration used [24]. Dietary fat, particularly short-chain saturated fatty acids and long-chain unsaturated fatty acids, favors lutein absorption [25]. The addition of 6% fat increases lutein deposition in chick tissues by three times [26]. Mycotoxins, such as aflatoxin or ochratoxin, decrease pigment absorption and reduce pigment accumulation in tissues [25].

It is known that over time, yolk and albumen weights change inversely, with greater water loss from the albumen being transferred to the yolk. This process affects weight and diameter, along with flattening and a decrease in yolk height [19]. Additionally, temperature and local conditions influence the release of carbon dioxide from the albumen into the environment, resulting in a lower percentage and weight of albumen as storage days progress [20].

The synthesis of yolk pigments is highly dependent on sources that provide carotenoids, with carotenoid-rich sources producing yolks with higher luminosity levels [27]. The intensification of red coloration in the yolks, with the inclusion of the (P/M) mixture and bell pepper in the diet, can be attributed to the presence of lutein, capsanthin, and zeaxanthin in bell pepper, which have red–orange pigmentation properties [28]. On the other hand, the increased intensity of yellow coloration is due to the inclusion of marigold, which contains xanthophylls in its composition [7].

Yolk coloration, as assessed by the parameters L* (brightness), a* (red/green intensity), and b* (yellow/blue intensity), was more intense in the groups that received pigments, especially for the birds fed marigold extract, which presented the highest concentration of beta-carotene in the yolk. These results corroborate the findings of [5], who reported that paprika and other plant extracts contain compounds that stimulate mucosal secretion and pancreatic enzyme activity, improving nutrient digestibility and potentially benefiting intestinal morphology. Microemulsified carotenoid pigments, both yellow and red, demonstrate enhanced bioavailability in laying hens, achieving equivalent yolk pigmentation at 20–30% lower dietary inclusion levels compared to conventional non-microemulsified pigments [29].

Other researchers [7,30] also reported that the use of natural pigments, such as paprika and marigold, did not significantly affect egg mass but improved yolk pigmentation. Yolk color is a crucial sensory characteristic for consumers, who often prefer yolks with a more orange hue [31]. Therefore, the addition of natural pigments may be an effective strategy to meet consumer expectations for high-quality products.

The results of the study on egg preservation indicated that the inclusion of natural pigments had a positive effect on maintaining egg quality during storage. Eggs from groups supplemented with natural pigments showed better results in terms of Haugh unit, albumen height, and yolk height throughout the storage period. These findings suggest that natural pigments may help preserve the internal quality of eggs during storage by reducing oxidative stress, which is crucial for maintaining egg quality.

The L*, a*, and b* values revealed that yolk color improved with natural pigment supplementation, even after 14 days of storage. These results corroborate the findings of [32], who suggested that water loss and accumulation on the yolk surface increase light reflection, resulting in higher L* values. The stability of yolk color during storage is essential to ensure consumer acceptance of eggs.

The β-carotene content in the yolk was affected by the inclusion of natural pigments. The yolk of birds fed diets containing marigold extract contained relatively high concentrations of beta-carotene, which aligns with the results obtained by [30,32,33]. These studies showed that natural pigments are more effective in yolk pigmentation than synthetic pigments. Physiologically, natural pigments, such as paprika and marigold, improve nutrient absorption and digestive function in birds. Oliveira [5] reported that these plant extracts stimulate mucosal secretion and pancreatic enzymes, increasing nutrient digestibility and potentially improving intestinal morphology.

According to [34], the metabolism of pigments, such as carotenoids, present in the feed occurs through their absorption in the intestinal lumen. These pigments are transported along with lipids and enter cells via lipoproteins present in the cell membrane [35]. Once absorbed, they accumulate in various lipid-rich tissues, particularly in the egg yolk [36]. In addition to their role in pigmentation, these pigments are strongly associated with a reduction in oxidative stress, through the action of lutein [37].

As the concentration of carotenoids increases, a more pronounced antioxidant effect is observed, neutralizing free radicals and, thus, reducing oxidative stress. This effect is particularly beneficial for embryonic development in broiler breeders, as well as contributing to higher quality in commercial poultry farming [38,39].

A high concentration of carotenoids in egg yolks is beneficial not only for color but also for the nutritional value of eggs [40]. Carotenoids are important antioxidants that can contribute to the health of the consumer, making eggs an even healthier food option [41]. Natural pigments are viable and effective alternatives for the egg production industry, meeting consumer demand for high-quality products with appropriate coloring [42].

These findings provide a solid foundation for the formulation of diets aimed at improving the quality of Japanese quail eggs during the laying phase.

5. Conclusions

The inclusion of paprika extract, marigold, or their combination in the diet of Japanese quail significantly enhanced yolk coloration and increased β-carotene content, without negatively affecting feed intake or egg production. However, worse feed conversion by mass was observed in the groups fed paprika, marigold, and the (P/M) mixture compared to the control group. While egg weight, albumen weight, and shell weight decreased in the supplemented groups, these natural pigments showed a positive impact in terms of pigmentation and color intensity. Additionally, pigments helped maintain egg quality during storage, particularly in terms of Haugh unit, albumen height, and yolk height, meeting consumer expectations for high-quality products. Future studies should focus on evaluating the antioxidant effects of these pigments in eggs, which may provide a deeper understanding of their potential benefits in terms of both nutritional quality and shelf-life enhancement.

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