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Evaluation of the fatty acid and carotenoid contents of the yolk and preservation of nutrients in eggs of Japanese quails fed different lipid sources*

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This study aimed to evaluate yolk lipid content, fatty acids, carotenoids, and the preservation of egg quality in quails fed diets containing different lipid sources. The experiment followed a completely randomized design with five treatments (soybean oil, corn oil, canola oil, sunflower oil, and poultry fat), 10 replicates, and seven birds per experimental unit, totaling 350 quails. Ether extract, carotenoid levels, fatty acid profile, and egg quality after 7 and 14 days of storage were evaluated in relation to lipid inclusions. Data were subjected to analysis of variance, and polynomial regressions were applied to estimate the effects of storage time. The effects of lipid sources were evaluated using Tukey's test at the 5% significance level. Lipid source had no effect on yolk ether extract content; however, quail fed soybean oil produced eggs with lower carotenoid levels. A significant effect (P≤0.05) of lipid source and of the interaction between lipid source and storage time was observed for egg quality variables, except yolk diameter. Different lipid sources did not influence the preservation of egg quality during storage, but they did affect carotenoid and fatty acid deposition in the yolk. The inclusion of sunflower oil improved the deposition of linoleic acid and β-carotene in egg yolks.

KEYWORDS: Shelf life / beta-carotene / lipid sources / omega-3 / omega-6

The different methods of producing quail eggs, especially the availability of processed eggs, increase consumption and production, resulting in a significant expansion of the Brazilian market and the demand for better quality final products. Eggs have great nutritional value because yolks are nuitrient rich. These nutrients are composed of unsaturated fatty acids, minerals, and vitamins that act beneficially in the human body [De Almeida *et al.* 2021].

Each component of the egg has specific functions in the body, and these can be modified by altering the birds' diet [Figueiredo *et al.* 2011]. In this context, nutrition directly influences product quality, and commonly used nutrients such as lipids can be strategic ingredients in production, as they provide a greater quantity of fatty acids in egg yolks [Hayat *et al*. 2010]. The demand for foods that improve health conditions, such as eggs enriched with polyunsaturated omega-3 fatty acids (ω-3), requires the supplementation of these acids in poultry diets.

Over time, the quality of eggs deteriorates as the storage period increases [Santos *et al.* 2021]. Therefore, egg-producing farms have sought alternatives to address the problem of egg deterioration over their shelf life. The inclusion of fatty acids in egg yolks can accelerate their deterioration over time due to the ease of oxidation, with the number of unsaturated molecules being a decisive factor for the reaction rate. Concentrations of unsaturated fatty acids, heat, oxygen, humidity, and pro-oxidant metals affect lipid peroxidation and, consequently, product quality [Moraleco *et al.* 2019].

Dietary lipids are commonly exposed to these pro-oxidant conditions during processing and storage, and the duration of exposure to these conditions determines the extent of peroxidation [Song and Shurson 2013]. During peroxidation, fatty acids are converted into various products, including peroxides, aldehydes, ketones, acids, esters, and other compounds [Rocha *et al*. 2013], which can negatively affect the physical, chemical, and organoleptic quality of eggs.

Thus, this study was conducted to characterize the lipid content, fatty acids, and carotenoids in the yolk and to determine the quality of eggs from Japanese quails fed different lipid sources.

Material and methods

The study was submitted and subsequently approved by the Research Ethics Committee under protocol number 16/2020. A total of 350 Japanese quail (*Coturnix japonica*) aged 85 days and with a 90% laying rate and an initial weight of 158.50 ±5.41 g were used. The plants were distributed in accordance with a completely randomized experimental design with 5 treatments (soybean oil, corn oil, canola oil, sunflower oil, and poultry fat) with 10 replicates and 7 Japanese quail at the peak laying time per experimental unit. The experiment lasted 84 days and was divided into 3 periods of 28 days each.

The diets were formulated based on corn and soybean meal without the inclusion of lipid sources and according to the food composition and nutritional requirements established by Rostagno *et al.* [2017], as shown in Table 1. The animal density per experimental unit was 178.5 cm²/bird. The experimental diets were isonutritive, differing only in the type and amount of lipids included to meet the birds' calculated energy requirement. The diets were offered ad libitum three times daily in galvanized metal trough feeders running the full length of the cages. The feeders were divided according to treatment and replication status. Water was also provided *ad libitum* to nipple-type drinkers.

Table 1. Nutritional and calculated composition of the experimental diets for Japanese quails in the production phase

Ingredients	Lipid sources				
	Soy	Corn	Canola	Sunflower	Poultry fat
Ground corn	56.291	56.291	56.291	56.291	56.291
Soy bran 45%	30.707	30.707	30.707	30.707	30.707
Inert	1.059	0.963	1.023	0.816	0.888
Lipid source	2.674	2.770	2.710	2.917	2.845
Limestone	6.950	6.950	6.950	6.950	6.950
Dicalcium phosphate	1.026	1.026	1.026	1.026	1.026
Salt	0.343	0.343	0.343	0.343	0.343
DL-methionine	0.417	0.417	0.417	0.417	0.417
L-lysine	0.323	0.323	0.323	0.323	0.323
Vitamin Premix[1]	0.100	0.100	0.100	0.100	0.100
Mineral premix[2]	0.100	0.100	0.100	0.100	0.100
	Nutritional composition				
EM (kcal/kg)	2800.00	2800.00	2800.00	2800.00	2800.00
Crude protein (%)	18.920	18.920	18.920	18.920	18.920
Digestible lysine (%)	1.149	1.149	1.149	1.149	1.149
Digestible Methionine+Cist (%)	0.942	0.942	0.942	0.942	0.942
Digestible Tryptophan (%)	0.186	0.186	0.186	0.186	0.186
Digestible Threonine (%)	0.733	0.733	0.733	0.733	0.733
Calcium (%)	2.990	2.990	2.990	2.990	2.990
Phosphorus available (%)	0.282	0.282	0.282	0.282	0.282
Sodium (%)	0.147	0.147	0.147	0.147	0.147

The composition of the mineral premix (kg/product) was as follows: copper (Min.) 7,000.0 mg; iron (Min.) 50.0 g; and iodine (Min.) 1500.0 mg. Manganese (Min.) 67.5 g. Zinc (Min.) 45.6 g. Vitamin Premix (kg/product): 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.) 5,000,000.0 IU. Vitamin B12 (Min.) 6,500.0 mcg. The vitamin B2 (Min.) 2000.0 mg. The vitamin B6 (Min.) 250.0 mg. Vitamin D3 (Min.) 1,850,000.0 IU. Vitamin E (Min.) 4500.0 IU. Vitamin K3 (Min.) 918.0 mg.

Temperature and relative humidity (RH) were recorded twice daily, at 8:00 a.m. and 4:00 p.m., using Novo Test TH802A thermohygrometers and dry- and wet-bulb thermometers placed at the centre of the shed at the birds' back height. The minimum temperature was 20.1±0.25°C, and the maximum temperature was 35.9±0.28°C, with a maximum RH of 78.9±1.8% and a minimum of 49.8±1.7%. Air conditioning and curtain control were performed based on daily temperature analysis. Sixteen hours of daily light was provided throughout the experimental period and was controlled by an automatic clock (timer). For the lipid characterization of the yolk, analyses of ether extract content, carotenoid content, and fatty acid content of the egg yolk and the diet were performed.

Ether extracts of the content of the yolk

Three hundred eggs were collected, 60 per treatment, on the same day of laying. Each replicate sample was composed of a pooled homogenate of six yolks. Each yolk replication sample was weighed, placed in an aluminium tray, and placed in a forced ventilation oven at 55°C for 72 hours. The samples were subsequently exposed to air to reach equilibrium at ambient temperature and humidity.

The plants were weighed to determine the dry matter content (DM), ground in Wiley mills with a 1 mm knife, and stored in a freezer at -20°C for laboratory analyses. The ether extract analyses were then carried out using a Soxhlet extractor following the methodology described by the Brazilian Animal Feeding Compendium (2009), with all the parameters expressed on a dry matter basis.

Carotenoid content

The carotenoid content was determined as described by Rodriguez Amaya [2010]. Carotenoid extraction was performed from 2 g of a sample taken from a pool of 3 eggs that had been previously macerated with chilled acetone, followed by filtration. The filtrate was transferred to a separation funnel, where petroleum ether was added, forming two layers: an upper layer of petroleum ether with carotenoids and a lower layer of water and acetone.

The lower layer was discarded, and to ensure the removal of acetone, the ether-carotenoid solution was washed 4 times with distilled water. The solution was then collected in a 50 mL volumetric flask and covered with aluminium foil to preserve the carotenoids. The extracts were evaluated on a spectrophotometer (Biochrom Libra S60PC model) at 450 nm. The results are expressed in mg equivalent of β-carotene/g of the sample.

Yolk and feed fatty acid contents

To evaluate the fatty acid profile of the yolks, six eggs were collected from each repetition on the last experimental day, forming a pool of six yolks, which were homogenized and frozen at -5°C in a freezer. Later, Lyophilization of yolks was carried out by sublimation. For fatty acid determination, the egg lipid fraction was

extracted following Bligh and Dyer [1959]. Sixty milligrams of the extracted lipid fraction was weighed and then subjected to methylation following Maia & Rodriguez-Amaya (1993) to prepare it for gas chromatography analysis. For the analysis of fatty acids, myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linolenic acid (C18:3) were extracted.

Fatty acids were identified by comparing retention times with standard methyl esters (Sigma-Aldrich) and quantified by area normalization, with results expressed as the percentage of each acid relative to the total fatty acid area. The esterification of lipids followed Hulan *et al*. [1989]. After esterification, the samples were analysed on a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector with manual injection, a capillary column (CARBOWAX), and H_2 as the carrier gas.

The fatty acid content was calculated through integration using specialized software connected directly to the gas chromatograph detector, ensuring precise quantification of the chromatographic peaks. This integration process enabled accurate determination of the relative proportions of fatty acids present in each sample.

In addition to yolk samples, representative samples of the experimental bird diets were collected and subjected to the same extraction and methylation procedures to characterize their fatty acid profiles to obtain a direct comparison between dietary lipid composition and deposition in egg yolks.

Table 2 presents the fatty acid composition of diets supplemented with different lipid sources for Japanese quail.

Table 2. Descriptive analysis of the fatty acid content of Japanese quail diets enriched with different lipid sources

Fatty acid profile (%)	Lipid sources				
	Soy	Corn	Canola	Sunflower	Poultry fat
Total Ac. fatty	95.47	95.44	95.28	96.96	96.14
C16:0 (Palmitic)	13.95	16.37	8.95	11.95	20.56
C16:1 (Palmitoleic)	0.53	0.59	0.67	0.53	6.94
C18:0 (Stearic)	7.13	5.97	4.13	6.13	8.03
C18:1w9 (Oleic)	26.67	34.63	55.81	26.78	42.54
C18:2w6 (Linoleic)	44.82	36.51	24.35	49.32	16.97
C18:3w3 (Linolenic)	2.12	1.12	1.12	2.03	0.89
C20:4w6 (Arachidonic)	0.13	0.13	0.13	0.11	0.10
C22:6w3 (Docosahexaenoic)	0.12	0.12	0.12	0.11	0.11

Egg quality. To verify the quality of plants under preservation, a completely randomized design was used with five treatments (lipid sources) and three storage periods (0 (fresh egg), 7, and 14 days) as repeated measures over time (5 × 3 factorial design), with 10 replications. Three eggs from each replicate were considered the experimental unit; a total of 450 eggs were analysed during each experimental period, and a total of 1350 eggs were evaluated; the eggs were kept at natural temperature throughout the entire storage period.

Egg storage (7 and 14 days) was carried out in a room with natural ventilation, free from direct sunlight, in dry and ventilated places, with minimum and maximum temperatures of 32.8±0.2°C and 21.9±0.15°C, respectively, a maximum relative

humidity (RH) of 69±1.3%, and a minimum RH of 41.5±1.5%. The eggs were identified according to their treatment and individually weighed using a semianalytical balance with a precision of 0.01 g. The quality of the eggs was evaluated following Moraleco *et al*. [2019].

Specific gravity. Eggs were immersed in eight saline solutions (NaCl) with densities from 1.065 to 1.100, increasing in 0.005 increments. The density at which the eggs float was considered their specific gravity following Castelló *et al*. [1989].

Yolk coloration. After the eggs were broken, the shell, yolk, and albumen were separated on a flat surface, and the yolk coloration was evaluated using a portable colorimeter model (Minolta CR 410). The luminosity (L*), redness (a*), and yellowness (b*) parameters were evaluated at three different points on the surface of the yolk. The egg colour was also evaluated using a La Roche colorimetric fan.

Height of yolk and albumen. Yolk and albumen heights and yolk diameter were measured with a calliper on a tripod; yolk height at the centre and albumen height 4 cm from the yolk. This analysis was performed by only one evaluator to obtain greater accuracy in the data.

Weight and percentage of yolk, albumen, and shell tissue. The yolks were separated from the albumen and weighed individually on a digital balance, and the weight of the albumen was obtained by the difference between the weight of the egg, the yolk weight, and the weight of the shell. The weight of the shell was obtained after washing and drying in an oven at 65°C for 72 hours. The percentages of shell, yolk, and albumen were obtained by dividing these components by the weight of the egg and multiplying these results by 100.

Shell thickness. After washing and drying, shell thickness was measured at three points with a Digimess precision micrometre (0.001 mm), and the mean of these measurements was recorded.

Haugh unit. The Haugh unit was measured using the mathematical equation described by Stadelman [1999], which correlates the weight of the egg with the height of the yolk or albumen.

Yolk indices. The yolk index was calculated as the ratio of yolk height to yolk diameter, according to Moraleco *et al*. [2019].

Statistical analysis

The statistical model used to analyse the data can be described in scalar notation as follows:

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ijk}$$

where:

Y_{ijk} – the observed response variable for the k-th observation of the i-th lipid source and j-th period;

μ – the overall mean;

τ_i – the fixed effect of the i-th lipid source (treatment);

β_j – the effect of the j-th period, included as a covariate;

$(\tau\beta)_{ij}$ – the interaction effect between the lipid source and period (when applicable);

ε_{ijk} – the residual error term, which is assumed to be normally and independently distributed with a mean of zero and constant variance.

To check statistical assumptions, the Shapiro-Wilk test was used to assess the normality of residuals, and Levene's test to evaluate homogeneity of variances.

Egg composition data were analysed by analysis of variance using the MIXED procedure of SAS (SAS 9.3). The period effect was included in the model as a covariate. When significant effects were found, the means were compared using Tukey's test at the 5% significance level.

Egg storage data were analysed by ANOVA using the MIXED procedure in SAS (v. 9.3), with the REPEATED statement to account for evaluation days as repeated measures over time. The period effect was included as a covariate. When significant interactions between lipid sources and time occurred, orthogonal polynomial regressions (linear and quadratic) were performed. For the main effects of lipid sources on egg quality, means were compared using Tukey's test. The significance level was set at 5% for all analyses.

Results and discussion

Lipid sources influenced the total fatty acids C16:0 (palmitic), C16:1 (palmitoleic), C18:1w9 (oleic), and C18:2w6 (linoleic) but did not affect the percentage of ether extracts in egg yolks. The inclusion of soybean oil in the diet resulted in a lower number of carotenoids in eggs than did the inclusion of sunflower oil, but eggs from birds fed corn, canola, or poultry fat lipid sources had similar beta-carotene contents (Tab. 3).

There was no effect ($P>0.05$) of the interaction between lipid source and storage time on the variables egg weight, yolk weight, shell weight, albumen weight, % yolk, % shell, or % albumen. However, there were isolated effects of storage period on yolk weight, albumen weight, % yolk, % shell, and % albumen. Yolk weight, % yolk, and % shell maintained an increasing linear relationship; that is, they increased with storage time. On the other hand, the albumen weight and percentage exhibited decreasing linear trends and decreased with increasing preservation period (Tab. 4).

For the variables related to egg colour, there was no interaction between the lipid source and storage period factor or between the lipid source factor and the individual factor. However, for the storage period factor, there was an isolated effect on the variables fan and colorimeter (L, A, and B), with a quadratic effect for the 0-, 7-, and 14-day storage times (Tab. 5). The L, A, and B parameters exhibited quadratic behaviour, as evidenced by the regression equations.

For the variables specific gravity, Haugh unit, yolk index, shell thickness, albumen height, yolk height, and yolk diameter, there was no interaction between the factors lipid source × storage period or the isolated lipid source factor. Only the albumen height was influenced by lipid inclusion. However, the storage period had an isolated effect on all the described variables (Tab. 6).

Linear and quadratic regression equations were generated for the variables that had significant effects on the storage time. For yolk weight, % yolk, % shell, and yolk diameter, there were linear increasing effects, where the values of these variables increased as the storage time increased. Albumen height, albumen weight, albumen percentage, yolk height, Haugh unit, and yolk index showed a linear decrease over time. Evaluating the specific gravity; colour parameters Leque, L, A, and B; and shell thickness, there was a quadratic effect concerning egg storage days, with maximum values occurring at Y=11.11 days, Y=8.08 days, Y=8.21 days, Y=8.44 days, Y=9.18 days, and Y=0.75 days, respectively.

Carotenoids are compounds of great dietary importance because they are precursors of vitamin A and play a role in the process of cellular protection and renewal. They also have a significant sensory function related to consumer attraction, as they provide colour to foods, which is one of the first noticeable characteristics of a product, given expectations of quality and flavour. Carotenoids are liposoluble pigments synthesized by plants and photosynthetic microorganisms that are generated by animals through their diet. Like all animals, birds metabolize carotenoids but cannot synthesize them and therefore require supplementation in their diet.

Table 3. Ether extract, beta-carotene, and fatty acid contents of quail egg yolk fed with different lipid sources

Variables	Lipid sources					SEM	P value
	soy	corn	canola	sunflower	poultry fat		
% EE of the yolk	56.335	56,573	56,422	57,593	56,277	0.379	0.137
Beta-carotene content	20.74^{B}	24.27^{AB}	25.13^{AB}	27.50^{A}	23.28^{AB}	0.657	0.017
Total Ac. fatty	96.11^{C}	96.09^{C}	97.15^{A}	97.03^{A}	96.71^{B}	0.085	<0.001
C16:0 (Palmitic)	25.08^{BB}	25.17^{B}	25.06^{B}	25.16^{B}	25.54^{A}	0.035	<0.001
C16:1 (Palmitoleic)	1.47^{B}	1.45^{B}	1.45^{B}	1.44^{B}	1.66^{A}	0.017	<0.001
C18:0 (Stearic)	9.26	9.29	9.29	9.28	9.28	0.007	0.752
C18:1w9 (Oleic)	45.75^{B}	45.51^{B}	46.70^{A}	45.59^{B}	45.64^{B}	0.085	<0.001
C18:2w6 (Linoleic)	13.93^{C}	14.06^{B}	14.03^{BC}	14.94^{A}	14.02^{BC}	0.070	<0.001
C18:3w3 (Linolenic)	0.20	0.21	0.20	0.19	0.20	0.001	0.676
C20:4w6 (Arachidonic)	0.19	0.19	0.19	0.19	0.19	0.001	0.950
C22:6w3 (Docosahexaenoic)	0.20	0.20	0.20	0.20	0.20	0.001	0.949

SEM – standard error of the mean; EE – ether extract.
^{ABC}Within rows means bearing different superscripts differ significantly at P<0.01.

Table 4. Weights and percentages of yolk, shell, and albumen of quail eggs fed lipid sources subjected to different storage times

Variables	Day	Lipid sources					Average	SEM	P value		
		soy	corn	canola	sunflower	poultry fat			source (S)	day (D)	S*D
Egg weight	0	11.00	10.91	10.80	11.00	10.71	10.88	0.031	0.591	0.212	0.820
	7	10.35	10.47	10.25	10.31	10.10	10.30				
	14	10.28	10.44	10.23	10.32	10.17	10.29				
	average	10.54	10.61	10.43	10.54	10.30	10.72				
Yolk weight	0	3.72	3.68	3.63	3.70	3.55	3.62	0.017	0.436	0.001	0.293
	7	3.50	3.72	3.63	3.61	3.63	3.66				
	14	3.88	3.90	3.99	3.94	3.95	3.93				
	average	3.70	3.77	3.75	3.75	3.71	3.74				
Shell weight	0	0.89	0.89	0.87	0.87	0.87	0.88	0.003	0.092	0.775	0.585
	7	0.89	0.91	0.88	0.87	0.87	0.88				
	14	0.88	0.88	0.88	0.89	0.86	0.88				
	average	0.88	0.89	0.88	0.88	0.86	0.90				
Albumen weight	0	6.40	6.38	6.26	6.38	6.32	6.35	0.031	0.0568	<0.0001	0.9263
	7	5.82	5.71	5.61	5.68	5.45	5.65				
	14	5.51	5.59	5.31	5.51	5.34	5.45				
	average	5.92	5.90	5.73	5.86	5.70	6.06				
% egg yolk	0	32.92	32.60	32.71	33.12	32.52	32.92	0.176	0.333	<0.001	0.556
	7	34.78	36.36	36.12	36.07	36.66	34.78				
	14	37.25	37.08	38.78	38.12	38.33	37.25				
	average	34.98	35.35	35.87	35.77	35.83	34.98				
% eggshell	0	8.15	8.24	8.09	8.00	8.22	8.14	0.026	0.560	<0.001	0.178
	7	8.56	8.70	8.52	8.45	8.61	8.57				
	14	8.58	8.45	8.64	8.63	8.56	8.57				
	average	8.43	8.46	8.42	8.36	8.45	8.43				
% albumen	0	58.73	59.05	59.03	58.74	59.38	58.99	0.196	0.393	<0.001	0.382
	7	56.51	55.04	55.10	55.87	54.60	55.42				
	14	54.02	54.32	52.40	53.61	53.07	53.48				
	average	56.42	56.14	55.51	56.07	55.68	55.96				

SEM – standard error of the mean.
Regression equation with a linear effect % egg yolk – $Y = 0.391x + 32.198$; P value <0.001; $R2 = 0.158$.
% eggshell – $Y = 0.031x + 8.177$; P value <0.001; $R2 = 0.146$.
% albumen – $Y = -0.419x + 59.689$; P value <0.001; $R2 = 0.175$.
Albumen height – $Y = -0.123x + 4.204$; P value <0.001; $R2 = 0.370$.
Albumen weight – $Y = -0.067x + 6.557$; P value < 0.001; $R2 = 0.144$.
Yolk weight – $Y = 0.023x + 3.596$; P value <0.001; $R2 = 0.048$.

According to Zaheer [2017], chicken eggs can be considered ideal carriers of biologically active carotenoids for human consumption. Moreover, moderate egg consumption is no longer associated with an increased risk of developing CHD in healthy individuals. Hens fed diets based on canola/corn oils with or without microalgae (*Nannochloropsis oculata*) produced eggs varying in carotenoid content and fatty acid composition of the yolk [Gładkowski *et al*. 2011]. This difference is associated with the greater amount of linoleic acid, which is consistent with the present research, where the addition of sunflower oil increased the carotenoid content and C18:2w6 (linoleic) fatty acid content.

Table 5. Yolk colour of quail eggs fed lipid sources subjected to different storage times

Variables	Day	Lipid sources					Average	SEM	P value		
		soy	corn	canola	sunflower	poultry fat			source (S)	day (D)	S*D
Fan	0	4.71	4.94	4.68	4.86	4.86	4.81	0.037	0.231	0.001	0.632
	7	5.13	5.16	4.98	5.30	4.92	5.10				
	14	4.55	4.75	4.73	4.93	4.93	4.78				
	average	4.79	4.95	4.80	5.03	4.90	4.90				
L*	0	55.68	55.89	55.64	55.52	56.11	55.77	0.260	0.877	<0.001	0.539
	7	64.69	65.92	66.35	64.63	66.22	61.24				
	14	61.86	61.54	61.15	60.57	61.38	61.30				
	average	60.74	61.12	61.05	60.24	61.24	61.53				
A*	0	-1.96	-1.85	-2.00	-1.78	-1.64	-1.84	0.035	0.389	<0.001	0.937
	7	-2.87	-3.14	-2.93	-2.98	-2.84	-2.95				
	14	-2.82	-2.32	-2.40	-2.46	-2.18	-2.44				
	average	-2.55	-2.43	-2.44	-2.40	-2.22	-2.41				
B*	0	36.01	37.10	36.54	36.34	37.68	36.73	0.225	0.083	<0.001	0.423
	7	44.06	46.49	44.31	45.71	45.23	45.16				
	14	44.30	45.04	44.05	44.13	44.87	44.48				
	average	41.46	42.88	41.63	42.06	42.59	42.49				

SEM – standard error of the mean.
Regression equation with quadratic effects Fan – Y = -0.006x^2 + 0.097x + 4.738; Y = 8.08 days; P value = 0.005; R2 = 0.013.
L – Y = -0.246x^2 + 4.043x + 51.868; Y = 8.21 days; P value <0.001; R2 = 0.441.
A – Y = 0.027x^2 - 0.456x - 1.442; Y = 8.44 days; P value <0.001; R2 = 0.042.
B – Y = -0.169x^2 + 3.076x + 33.543; Y = 9.18 days; P value < 0.001; R2 = 0.819.

Another important point related to the content of carotenoids in egg yolk is the natural antioxidant effect of pigments. Egg farms have sought alternatives to increase the shelf-life of eggs and reduce product deterioration. The use of antioxidants is a reliable option since they increase the shelf-life of eggs destined for consumers. Therefore, exploring dietary enrichment sources is a way to add value to poultry products, ensuring improved nutritional quality.

Egg yolks from birds fed canola and sunflower lipid sources presented greater total fatty acid quantities than did those from birds fed soy, corn, or poultry fat sources. Poultry fat provided more palmitic acid (C16:0) and palmitoleic acid (C16:1) to the egg yolk. The levels of C18:0 (stochastic), C18:3w3 (linolenic), C20:4w6 (arachidonic), and C22:6w3 (docosahexaenoic) were similar between the treatments. The addition of canola oil provided a greater quantity of oleic acid (C18:1w9), whereas sunflower oil increased the amount of linoleic acid (C18:2w6) in the yolks.

Among the monounsaturated fatty acids, oleic acid (C18:1) is present in greater quantity in egg yolk, whereas among the polyunsaturated fatty acids (PUFAs), linolenic acid is considered the most important (ω-3) because it is an essential fatty acid and is important for inclusion in the diet. The lipid fraction of eggs can be altered by altering birds'diet. There are two classes of PUFAs, n-6 and n-3, and n-3 PUFAs are useful for optimal human health. The three main n-3 PUFAs are alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA).

Table 6. Quality indices of eggs from quails fed lipid sources subjected to different storage times

Variables	Day	Lipid sources					Average	SEM	P value		
		soy	corn	canola	sunflower	poultry fat			source (S)	day (D)	S*D
Specific gravity	0	1.073	1.074	1,070	1.071	1.073	1.072	0.001	0.054	<0.001	0.057
	7	1.065	1.065	1.065	1.065	1.065	1.065				
	14	1.065	1.065	1.065	1.065	1.065	1.065				
	average	1.067	1.068	1.066	1.067	1.067	1.07				
Haugh unit	0	87.86	86.93	87.41	87.84	89.24	87.86	0.220	0.118	<0.001	0.597
	7	82.12	81.14	82.77	81.65	82.47	82.03				
	14	78.39	77.93	76.69	77.39	78.50	77.78				
	average	82.79	82.00	82.29	82.29	83.41	82.56				
Shell thickness	0	0.21	0.22	0.21	0.21	0.21	0.21	0.001	0.222	<0.001	0.887
	7	0.20	0.21	0.20	0.20	0.20	0.20				
	14	0.22	0.22	0.21	0.22	0.21	0.22				
	average	0.21	0.21	0.21	0.21	0.21	0.21				
Albumen height	0	4.10	4.01	4.08	4.19	4.39	4.15	0.034	0.195	<0.001	0.766
	7	3.14	3.05	3.24	3.09	3.19	3.14				
	14	2.59	2.57	2.36	2.47	2.60	2.52				
	average	3.28	3.21	3.23	3.25	3.39	3.27				
Gem height	0	10.44	10.59	10.55	10.42	10.59	10.52	0.060	0.026	<0.001	0.121
	7	8.50	8.21	8.25	8.17	8.24	8.27				
	14	6.83	6.75	6.52	6.53	6.67	6.66				
	average	8.59^{A}	8.51^{AB}	8.44^{AB}	8.37^{B}	8.50^{AB}	8.54				
Bud diameter	0	23.40	23.28	23.41	23.31	23.16	23.31	0.121	0.616	<0.001	0.804
	7	26.39	26.38	26.08	26.13	25.85	26.17				
	14	30.46	30.89	31.03	30.64	30.69	30.74				
	average	26.75	26.85	26.84	26.69	26.57	26.72				

SEM – Standard error of the mean.
^{AB}Within rows means bearing different superscripts differ significantly at P<0.01.
Regression equation with quadratic effects Gravity – Y = 0.00009x^2 - 0.002x + 1.074; Y = 11.11 days; P value <0.001; R2 = 0.353.
Shell thickness – Y = -0.0002x^2 + 0.0003x + 0.0002; Y = 0.75 days; P value <0.001; R2 = 0.062.
Regression equation with linear effects: Bud Diameter – Y = 0.574x + 22.52; P value <0.001; R2 = 0.708. Gem Height – Y = -0.295x + 10.702; P value <0.001; R2 = 0.753. Haugh unit – Y = -0.770x + 88.19; P value <0.001; R2 = 0.386.

Neijat *et al*. [2016] reported an increase in polyunsaturated fatty acids in chicken eggs when flaxseed oil and DHA microalgae (docosahexaenoic acid) were included in the diet. This was confirmed in the present study, as variations in dietary fatty acid content resulted in differences in yolk fatty acid composition. Canola oil promoted greater oleic acid deposition, while sunflower oil increased linoleic acid content, an essential fatty acid. Linolenic acid was found in similar amounts across treatments. Linoleic and linolenic acids serve as precursors for the omega-6 and omega-3 fatty acid families, respectively.

These fatty acids contain more carbons and double bonds, making them biologically active and essential for bodily functions beyond serving as an energy source. Linoleic acid, for example, acts on cell membrane receptors and influences enzymatic activity. Thus, assessing fatty acid profiles in the diet is an important approach for determining food composition, as verified in this study (Tab. 2).

Eggs are valuable sources of essential fatty acids, which are important for improving health. However, the fatty acids in egg yolks are susceptible to lipid oxidation. Such oxidative processes in eggs can become a problem in the production of eggs enriched with polyunsaturated fatty acids [Pereira *et al*. 2019].

Nutrient deterioration is common in foods, and lipid oxidation is considered the most important process because it can affect quality, especially aroma, flavour, and nutritional value, and can produce toxic compounds that cause rancidity, altering the flavour of the food [Pereira *et al*. 2019]. In this sense, the evaluation of nutrient preservation in eggs of birds fed diets containing different sources of lipids is an area that should be studied.

Owing to the high concentration of double-bond fatty acids in eggs, the yolk becomes sensitive to lipid oxidation, and this process is responsible for the formation of peroxides that cause oxidative degradation of the yolk. To delay this natural deterioration process, it is necessary to maintain eggs in a low-temperature environment with controlled humidity. The lower the temperature and the higher the humidity are, the longer the freshness characteristics are preserved in the egg [Figueiredo *et al*. 2011].

To maintain or increase the durability of eggs, especially with respect to lipid oxidation, and to favour the maintenance of the fatty acid profile, a common practice is the use of natural antioxidants such as annatto, turmeric, paprika, and marigold flowers [Moraleco *et al.* 2019]. In this way, it was verified that lipid sources can influence the carotenoid content of egg yolks and, consequently, the storage time of this product.

These reactions promote the liquefaction of albumen and, consequently, the release of carbon dioxide, which diffuses through the pores of the shell and is lost into the environment [Rocha *et al.* 2013]. Thus, albumen weight and percentage decreased, while yolk weight increased due to water transfer from the albumen to the yolk, enlarging its size and proportion.

This finding is consistent with that of Santos *et al*. [2021], who reported that as air enters through an eggshell, the degradation of albumen begins, altering the consistency of the albumen and the integrity of the chalazae. Subsequently, the yolk shifts and the vitelline membrane ruptures, allowing albumen degradation products to enter and alter its composition.

The results obtained in the present study reinforce those of Santos *et al.* [2021], who reported that the percentage of albumen decreases with time because of the liquefaction of the albumen in conjunction with the gas exchange of the eggs with the environment.

According to Giampietro-Ganeco *et al*. [2012], egg pigmentation is stable when eggs are refrigerated and decreases when they are stored at room temperature. However, in this study, the L, A*, and B* values were greater. On the basis of the derivative of the regression equations, the highest colour indices occurred at approximately 8-9 days of storage (Variables Leque, L, A, B: Y = 8.08 days, Y = 8.21 days, Y = 8.44 days, Y = 9.18 days, respectively), indicating that over time, the eggs lost water through gas

exchange with the environment, leading to more concentrated pigment components in the yolk (carotenoids). However, after nine days of storage, these values decreased. This can be explained by the findings of Moraleco *et al.* [2019], who reported that food oxidation destroys vitamins, essential fatty acids, proteins, and pigments, reducing the luminous content over time.

In this study, the effects of storage time and the presence of various lipid sources on egg quality were investigated. We found refrigeration maintains colour stability during storage and prevents chemical reactions that could negatively affect egg quality. In contrast, when eggs were stored at room temperature, the degree of colour preservation decreased over time.

Lipid sources did not directly influence the preservation process during storage. Lipid oxidation defines a large part of the shelf life and quality of eggs. This process usually occurs during storage and leads to the denaturation of fat-soluble vitamins and essential fatty acids, as well as the generation of undesirable products from a sensory standpoint.

When the lipid profile of eggs is modified, there is concern about the shelf-life of this product and the need to use antioxidants in diets to improve preservation. However, no differences among treatments or lipid source interactions were observed in egg quality deterioration; time was the only influencing factor [Qin *et al*. 2018]. The incorporation of oils and fats into the diet of Japanese quail may have practical value for manipulating the quality of egg yolks, but it does not influence quality preservation for up to 14 days of storage.

In addition to meeting energy requirements, essential fatty acids, and health, lipid sources can also positively affect the fatty acid composition of quail eggs, especially the w3/w6 ratio, making them healthier foods for human nutrition. Thus, given that there is a change in the fatty acid profile of egg yolks from birds supplemented with different lipid sources in the diet and that these eggs are more susceptible to oxidation, eggs must be stored in chilled environments to ensure sensory and nutritional quality.

Conclusions

Different lipid sources do not interfere with the preservation of unrefrigerated egg quality but they directly influence the deposition of carotenoids and fatty acids in the yolk of eggs. The inclusion of sunflower oil improved the content of linoleic acid and beta-carotene in egg yolks.

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Disclosure statement

The authors report that there are no competing interests to declare.

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