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Performance, quality, and oxidative stability during storage of eggs from white laying hens fed diets supplemented with carotenoids

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ABSTRACT - The aim of the study was to evaluate the inclusion of carotenoids in the diet of layers on the production performance, quality and oxidative stability of eggs during storage. A total of 240 Dekalb White laying hens, 76 weeks of age, were used. The hens were allocated in completely randomized design with eight diets (control diet (CD); astaxanthin (AXT), 10 mg kg^{-1}; lutein (LUT), 10 mg kg^{-1}; lycopene (LYC), 10 mg kg^{-1}; AXT + LUT, 10 mg kg^{-1} each; AXT + LYC, 10 mg kg^{-1} each; LUT + LYC, 10 mg kg^{-1} each; and AXT + LUT + LYC, 10 mg kg^{-1} each), five replicates, and six birds per experimental unit. Egg quality and oxidative stability were evaluated using a randomized 8 × 3 factorial design (diets × storage period: fresh, 15, and 30 days) with repeated measures. Eggs were stored either at room temperature (RT, 25 °C) or under refrigeration (RF, 8 °C) for the evaluation of egg quality and lipid stability. Dietary supplementation with astaxanthin (AXT; 10 mg kg^{-1}), lutein (LUT; 10 mg kg^{-1}), and lycopene (LYC; 10 mg kg^{-1}), either individually or in combination, did not affect the zootechnical performance or overall egg quality of laying hens. However, AXT supplementation improves the oxidative stability of eggs compared with LYC, suggesting a greater protective effect against oxidative deterioration. Combined supplementation with AXT + LUT, AXT + LYC, and LUT + LYC enhances yolk pigmentation, whereas the simultaneous inclusion of all three carotenoids reduces pigmentation intensity. Regardless of dietary treatment, egg storage for up to 30 days at room temperature (25 °C) or under refrigeration (8 °C) negatively affects the internal quality of eggs.

Keywords: astaxanthin, color, lutein, lycopene, storage

1. Introduction

Eggs provide several health benefits throughout the human lifespan (Puglisi and Fernandez, 2022). They are considered one of the most nutritionally complete foods for human consumption (Zaheer, 2015), containing several essential nutrients, particularly vitamin D, vitamin B12, selenium, choline, and essential amino acids (Rafed et al., 2024). To fully capitalize on its nutritional potential, preserving eggs during the marketing period is crucial, given the weeks that may pass between laying, acquisition, and preparation. Extended periods negatively impact egg quality, with a continuous decline after laying (Carvalho et al., 2007).

A promising alternative is the use of technologies aimed at maintaining egg quality after laying, thereby extending the shelf life of eggs and egg-derived products. In the context of animal production and food technology, considerable research has focused on antioxidants and their effects on the shelf life and nutritional quality of food (Hayat et al., 2010). Eggs contain substantial amounts of unsaturated fatty acids, which highly susceptible to lipid oxidation processes, limiting their conservation capacity (Pita et al., 2004). Currently, there is a tendency to substitute synthetic antioxidants with natural alternatives due to concerns about liver alterations the formation of cancer cells associated with the exposure of animals to synthetic compounds (Jardini and Mancini Filho, 2007).

Carotenoids, when used as feed additives in poultry diets, enhance yolk color and increase the boost antioxidant capacity, protecting eggs from the harmful effects of reactive oxygen species and free radicals (Nabi et al., 2020). In terms of oxygen-sequestration ability of carotenes and xanthophylls, lycopene, AXT or canthaxanthin, β-carotene or bixin, lutein, and crocin follow an ascending order (Fontana et al., 2000). The most commonly used carotenoids are lycopene, lutein, astaxanthin, β-carotene, fucoxanthin, and canthaxanthin (Darvin et al., 2022). Carotenoids not only intensify egg yolk pigmentation but also exhibit multiple biological roles that contribute to therapeutic effects, including anticancer, immunomodulatory, anti-inflammatory, antibacterial, antidiabetic, and neuroprotective activities (Nabi et al., 2020).

Utilizing antioxidants, including carotenoids such as AXT, LUT, and lycopene, represents an alternative strategy to strengthen the enzymatic antioxidant system against free radical attacks. Moreover, synergistic interactions among antioxidants may enhance the body's defense mechanisms and help prevent excessive free radical production, as these compounds possess distinct characteristics and modes of action. This may contribute to the inhibition of lipid peroxidation in egg yolk, thereby improving the oxidative stability of eggs (Panaite et al., 2021). Therefore, this study aimed to evaluate the effects of dietary carotenoid supplementation on the performance, egg quality, and oxidative stability of eggs from white laying hens stored for up to 30 days either at room temperature (RT) or under refrigeration (RF).

2. Material and methods

2.1. Ethical matters

Research on animals was conducted according to guidelines of the Ethics Committee on Animal Use of the Universidade Estadual de Mato Grosso do Sul (UEMS) (protocol number 038/2017).

2.2. Experiment design and treatments

The experiment was conducted at the Poultry and Animal Products Quality Laboratory of the Universidade Estadual de Mato Grosso do Sul, Aquidauana, MS, Brazil (20°20' S, 55°48' W, altitude of 174 m). The study involved 240 Dekalb White-strain laying hens, aged 76 weeks, evaluated over a 112-day experimental period divided into four 28-day cycles. The birds were housed in galvanized wire cages measuring 25 × 40 × 45 cm in a conventional laying house. A continuously supplied water trough was positioned along the front of the cages and cleaned daily. Feed was supplied *ad libitum* at 08:00 and 16:00 h in feeders located below the water troughs.

The lighting program adopted was 17 h daily (natural + artificial lighting). Thermal conditions in the aviary were assessed using a thermohygrometer at 08:00 and 16:00 h, with records of average (30.6 °C), minimum (24.0 ± 0.8 °C), and maximum temperatures (35.8 ± 2.7 °C), as well as relative humidity (83.8 ± 6.0%).

A completely randomized design was adopted with eight diets, five replications, and six birds per experimental unit. Diets included: control diet (CD) without carotenoids, CD with 10 mg kg^{-1} of AXT, CD with 10 mg kg^{-1} LUT, CD with 10 mg kg^{-1} LYC, CD with 10 mg kg^{-1} of AXT and 10 mg kg^{-1} of LUT, CD with 10 mg kg^{-1} of AXT and 10 mg kg^{-1} of LYC, CD with 10 mg kg^{-1} of LUT and 10 mg kg^{-1} of LYC, and CD supplement with 10 mg kg^{-1} of AXT, 10 mg kg^{-1} of LUT and 10 mg kg^{-1} of LYC. The experimental diets, based on corn and soybean meal, were formulated to be isoenergetic and isonutritive (Table 1) to meet the nutritional requirements of the hens, according to the strain manual (Dekalb White, 2009) and Rostagno et al. (2017). No antioxidants were included in the CD. Carotenoids were incorporated into the feed in powder form.

Table 1 - Percentage and calculated compositions of the control diet[1]

Ingredient	Control diet
Corn	62.00
Soybean meal, 46.5%	22.91
Soybean oil	2.07
Calcitic limestone	10.30
Dicalcium phosphate	1.58
L-Lysine HCl, 78.5%	0.10
DL-Methionine, 99%	0.33
L-Threonine, 98%	0.06
Salt	0.50
Mineral and vitamin supplement[2]	0.10
Calculated values	
ME (kcal kg^{-1})	2,900
CP (%)	15.61
Digestible methionine + cystine (%)	0.76
Disgetible threonine (%)	0.60
Digestible lysine (%)	0.78
Calcium (%)	4.43
Available phosphorus (%)	0.37
Sodium (%)	0.21

[1] Each carotenoid was added to the control diet in an amount of 10 mg kg^{-1} individually (Astaxanthin - AXT, Lutein - LUT, Lycopene - LYC) or combined (AXT + LUT, AXT + LYC, LUT + LYC and AXT + LUT + LYC).

[2] Composition per kg of feed: vitamin A, 9,637 IU; vitamin D3, 2,409 IU; vitamin E, 36.1 IU; vitamin K3, 1.93 mg; vitamin B1, 2.59 mg; vitamin B2, 6.44 mg; nicotinic acid, 39.2 mg; pantothenic acid, 12.95 mg; vitamin B6, 3.614 mg; vitamin B12, 0.0159 mg; folic acid, 0.903 mg; biotin, 0.090 mg; choline, 392 mg; Cu, 8.37 mg; Fe, 41.68 mg; I, 0.843 mg; Mn, 58.36 mg; Se, 0.250 mg; Zn, 54.21 mg.

2.3. Evaluation of performance and quality characteristics of eggs

Animal performance was evaluated through feed intake (g hen^{-1} day^{-1}), egg production (%), egg mass (g hen^{-1}), and feed conversion ratio (kg kg^{-1} and kg dz^{-1}). For egg quality assessment during the last four days of each cycle, six eggs by diet were randomly selected to measure average weight (g) and specific gravity (g cm^{-3}). Of these, three eggs were used to evaluate shell thickness, shell percentages, albumen, yolk, yolk pH, and albumen pH. The remaining eggs were used to measure the Haugh Unit (HU), yolk index (mm), and raw yolk color.

Using data from the total weight and number of eggs, average egg weight was calculated. For composition and pH analysis, eggs were individually weighed and broken into non-toxic polystyrene containers, previously identified and weighed, with complete separation of the albumen, yolk, and shell. The weight and percentage of each constituent relative to egg weight were calculated. The pH of the albumen and yolk was determined with a bench-top pH meter (Hanna Instruments®). Specific gravity was determined by the water displacement method (Hamilton, 1982).

Eggs were broken onto a smooth glass surface, albumen and yolk heights (mm) were measured using a digital caliper. Using albumen height (mm) and egg weight (g), HU values were calculated using the equation described by Silversides and Budgell (2004):

$$HU = 100\log(H + 7.75 - 1.7W^{0.37}),$$

in which H = albumen height (mm) and W = egg weight (g).

The yolk diameter was measured horizontally with a digital caliper (± 0.05 mm), and the yolk index (mm) (height/diameter) was calculated based on mean values. Raw yolk color analysis was evaluated using a DSM colorimetric fan (Yolk Color Fan®) on a scale from 1 to 15, reflecting varying yellow tones. Shells were washed, dried at RT for 48 hours, and subsequently weighed to determine external quality using shell weight (g) and thickness (mm). Shell thickness, including membranes, was measured at four distinct equatorial points using a digital micrometer (± 0.001 mm) and averaged per experimental unit.

To evaluate egg quality and oxidative stability, a randomized design was adopted in an 8 × 3 factorial arrangement (experimental diets × storage periods), with repeated measures, using two eggs diet^{-1} day^{-1}. Internal quality parameters, including HU, yolk index, yolk color, and albumen and yolk pH, were evaluated for each storage period at RT (25 °C) and RF (8 °C). Lipid oxidation was assessed using the TBARS method according to the methodologies described by Ramanathan and Das (1992) and Vyncke (1970), using three eggs diet^{-1} day^{-1} for each storage period.

2.4. Statistical analysis

The data were submitted to the Shapiro-Wilk normality test. For normally distributed residues, analysis of variance was applied, and the means were compared using the Tukey test. The TBARS variable, which did not present a normal distribution, was analyzed using a generalized linear model with a gamma distribution and inverse link function, and means were compared using the t-test. Statistical analyses were performed using the R software. The following mathematical model was adopted:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

in which Y_{ij} = dependent variable, μ = mean of the variable, α_i = effect of diets, and ε_{ij} = random error.

For quality analyses, the experimental design utilized was completely randomized in a 8 × 3 factorial scheme. The statistical model utilized was:

$$Y_{ijk} = \mu + D_i + S_j + (DS)_{ij} + \varepsilon_{ijk},$$

in which Y_{ijk} = represents the observation obtained from hens subjected to the i-th diet (i = 1-8) and j-th storage period (1-3); μ = is the overall mean; D_i = is the effect of diets; S_j = is the effect of storage period; $(DS)_{ij}$ = is the interaction effect between diets and storage period; and ε_{ijk} = is the random error associated with each observation. Statistical significance was declared at $P<0.05$.

3. Results

There was no significant effect ($P>0.05$) on the zootechnical performance of laying hens with the inclusion of AXT, LUT, and LYC, either individually or in combination (Table 2). With respect to egg quality variables, most showed no differences among experimental diets, except for raw yolk color. LYC supplementation and the combinations of AXT and LUT, AXT and LYC, and LUT and LYC resulted in more pigmented yolks compared to the CD (Table 3). No significant effects of carotenoid inclusion, whether individually or combined, were observed for the percentages of shell, albumen, and yolk, as well as specific gravity, shell thickness, and percentage of shell per surface of area in the diets of laying hens (Table 4). Raw egg yolks from hens fed the combination containing AXT and LUT, AXT and LYC, and LUT and LYC were more pigmented than those from hens on the combination of AXT, LUT, and LYC (Table 5). Differences in the concentration of malonaldehyde (MDA) and raw yolk color were observed between experimental diets for RF eggs (8 °C) (Table 6). Yolk indexes for eggs from laying hens fed the CD supplemented with AXT and LUT, as well as AXT and LYC, decreased after 15 days of storage (Table 7).

Table 2 - Animal performance, laying percentage and egg mass of white laying hens fed with diets supplemented with different carotenoids

Variable	FI ($g\ hen^{-1}\ day^{-1}$)	FC ($kg\ kg^{-1}$)	FC ($kg\ dz^{-1}$)	LP (%)	EM ($g\ hen^{-1}$)
Control diet	105.21	1.80	1.43	90.57	60.06
AXT	104.91	1.78	1.41	88.97	58.59
LUT	104.24	1.79	1.43	89.32	59.35
LYC	103.04	1.85	1.49	82.73	55.00
AXT + LUT	105.22	1.86	1.45	86.93	56.87
AXT + LYC	104.96	1.81	1.43	90.34	59.02
LUT + LYC	108.00	1.87	1.51	87.45	58.65
AXT + LUT + LYC	105.46	1.92	1.52	87.67	56.79
Mean	105.46	1.81	1.45	87.99	58.01
CV (%)	2.65	5.36	4.25	5.01	6.24
P-value	0.41	0.76	0.29	0.16	0.40

FI: feed intake; FC: feed conversion; LP: laying percentage; EM: egg mass; AXT: astaxanthin; LUT: lutein; LYC: lycopene; AXT + LUT: astaxanthin + lutein; AXT + LYC: astaxanthin + lycopene; LUT + LYC: lutein + lycopene; AXT + LUT + LYC: astaxanthin + lutein + lycopene.

Table 3 - Egg quality of white laying hens fed diets supplemented with different carotenoids

Variable	EW (g)	HU	YI (mm)	RYC
Control diet	66.15	94.36	0.411	5.56b
AXT	65.44	93.13	0.423	6.01ab
LUT	66.58	95.21	0.414	6.20ab
LYC	66.89	93.13	0.419	6.55a
AXT + LUT	65.59	94.85	0.427	6.51a
AXT + LYC	65.79	93.79	0.423	6.56a
LUT + LYC	67.40	93.58	0.415	6.33a
AXT + LUT + LYC	64.58	94.65	0.419	5.96ab
Mean	66.05	94.08	0.418	6.21
CV (%)	2.59	2.04	4.03	5.43
P-value	0.25	0.54	0.81	<0.05

EW: egg weight; UH: Haugh unit; YI: yolk index; RYC: raw yolk color; AXT: astaxanthin; LUT: lutein; LYC: lycopene; AXT + LUT: astaxanthin + lutein; AXT + LYC: astaxanthin + lycopene; LUT + LYC: lutein + lycopene; AXT + LUT + LYC: astaxanthin + lutein + lycopene.
Different letters in the same column differ at $P<0.05$.

Table 4 - Percentages of shell, albumen and yolk, specific gravity and shell thickness of eggs from white laying hens fed diets supplemented with different carotenoids

Variable	Shell	Albumen (%)	Yolk (%)	SG ($g\ cm^{-3}$)	ST (mm)	PSSA ($mg\ cm^{-2}$)
Control diet	8.91	73.99	24.85	1.083	0.322	75.28
AXT	8.82	73.67	25.09	1.082	0.321	78.05
LUT	8.67	73.75	25.28	1.083	0.325	75.48
LYC	8.46	73.87	25.27	1.083	0.327	75.44
AXT + LUT	8.97	73.37	25.16	1.082	0.319	74.82
AXT + LYC	8.76	73.14	25.58	1.083	0.325	75.72
LUT + LYC	8.63	73.00	25.86	1.083	0.324	75.91
AXT + LUT + LYC	8.80	72.88	26.24	1.083	0.316	75.78
Mean	8.75	73.45	25.41	1.082	0.322	75.81
CV (%)	5.01	1.42	4.68	0.23	4.26	3.33
P-value	0.67	0.58	0.65	0.99	0.93	0.64

SG: specific gravity; ST: shell thickness; PSAS: percentage of shell per surface of area; AXT: astaxanthin; LUT: lutein; LYC: lycopene; AXT + LUT: astaxanthin + lutein; AXT + LYC: astaxanthin + lycopene; LUT + LYC: lutein + lycopene; AXT + LUT + LYC: astaxanthin + lutein + lycopene.

Table 5 - Malonaldehyde concentration, egg weight, Haugh unit, yolk index, pH of albumen and yolk, raw yolk color, of eggs stored for different periods of storage at room temperature, from white laying hens fed diets supplemented with different carotenoids

Variable	MDA ($mg\ kg^{-1}$)	EW (g)	HU	YI (mm)	pH		RYC
					Yolk	Albumen	
Control diet	0.174b	62.58	62.76	0.307	6.38	8.78	6.07ab
AXT	0.164b	59.54	54.91	0.280	6.42	8.91	6.40ab
LUT	0.194ab	61.86	59.58	0.305	6.19	8.82	6.25ab
LYC	0.262a	60.21	53.24	0.281	6.37	8.92	6.40ab
AXT + LUT	0.196ab	63.45	57.58	0.288	6.37	8.89	6.75a
AXT + LYC	0.198ab	61.18	58.56	0.285	6.45	8.95	6.69a
LUT + LYC	0.201ab	64.03	58.21	0.290	6.48	8.86	6.69a
AXT + LUT + LYC	0.228ab	59.71	57.70	0.298	6.31	8.91	5.84b
Storage period (SP; days)							
Fresh	0.199b	66.15a	91.60a	0.414a	6.12b	8.00b	5.39c
15	0.201b	61.71b	44.93b	0.276b	6.24b	9.30a	6.54b
30	0.207a	58.22c	41.06b	0.217c	6.75a	9.33a	7.00a
Mean	0.202	61.57	57.82	0.292	6.37	8.88	6.39
P-value*							
Diet	0.03	0.38	0.29	0.11	0.82	0.77	0.03
SP	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Diet × SP	0.53	0.94	0.46	0.59	0.86	0.95	0.97

MDA: malonaldehyde concentration; EW: egg weight; HU: Haugh unit; YI: yolk index; pH: pH values of yolk and albumen; RYC: raw yolk color; AXT: astaxanthin; LUT: lutein; LYC: lycopene; AXT + LUT: astaxanthin + lutein; AXT + LYC: astaxanthin + lycopene; LUT + LYC: lutein + lycopene; AXT + LUT + LYC: astaxanthin + lutein + lycopene.
* Values with P>0.05 present normal distribution by the Shapiro Wilk test.
Different letters in the same column differ at P<0.05.

Table 6 - Malonaldehyde concentration, egg weight, Haugh unit, yolk index, pH of albumen and yolk, raw yolk color, of eggs stored for different periods of storage under refrigeration, from white laying hens fed diets supplemented with different carotenoids

Variable	MDA ($mg\ kg^{-1}$)	EW (g)	HU	YI (mm)	pH		RYC
					Yolk	Albumen	
Control diet	0.146b	61.24	79.15	0.416	6.16	8.66	6.00bc
AXT	0.151b	65.39	80.27	0.416	6.30	8.76	6.30abc
LUT	0.189ab	59.99	81.40	0.413	6.22	8.79	6.08bc
LYC	0.228a	59.86	81.54	0.409	6.35	8.74	6.50ab
AXT + LUT	0.194ab	64.86	82.97	0.407	6.58	8.78	6.82a
AXT + LYC	0.206ab	63.95	80.05	0.405	6.10	8.80	6.72a
LUT + LYC	0.226a	65.56	80.29	0.404	6.28	8.69	6.81a
AXT + LUT + LYC	0.246a	62.88	84.73	0.404	6.60	8.73	5.79c
Storage period (SP; days)							
Fresh	0.194	66.15a	91.60a	0.421a	6.12b	8.00b	5.59b
15	0.197	63.85a	79.98c	0.411ab	6.30ab	9.06a	6.75a
30	0.204	59.99b	79.96b	0.395b	6.56a	9.17a	6.88a
Mean	0.198	62.96	81.30	0.409	6.32	8.74	6.37
P-value*							
Diet	0.04	0.13	0.48	0.86	0.19	0.88	0.02
SP	0.17	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Diet × SP	0.50	0.94	0.95	0.019	0.11	0.99	0.41

MDA: malonaldehyde concentration; EW: egg weight; HU: Haugh unit; YI: yolk index; pH: pH values of yolk and albumen; RYC: raw yolk color; AXT: astaxanthin; LUT: lutein; LYC: lycopene; AXT + LUT: astaxanthin + lutein; AXT + LYC: astaxanthin + lycopene; LUT + LYC: lutein + lycopene; AXT + LUT + LYC: astaxanthin + lutein + lycopene.
* Values with P>0,05 present normal distribution by the Shapiro Wilk test.
Different letters in the same column differ at P<0.05.

Table 7- Yolk index of eggs from white laying hens fed diets with different sources of carotenoids stored up to 30 days under refrigeration

Diet	Storage period (days)		
	Fresh	15	30
Control diet	0.448a	0.400b	0.393b
AXT	0.411	0.397	0.402
LUT	0.421	0.411	0.413
LYC	0.423	0.410	0.383
AXT + LUT	0.449a	0.399b	0.373b
AXT + LYC	0.442a	0.393b	0.391b
LUT + LYC	0.406	0.421	0.384
AXT + LUT + LYC	0.431	0.409	0.404
Mean	0.411	0.392	0.422

AXT: astaxanthin; LUT: luten; LYC: lycopene; AXT + LUT: astaxanthin + Lutein; AXT + LYC: astaxanthin + lycopene; LUT + LYC: lutein + lycopene; AXT + LUT + LYC: astaxanthin + lutein + lycopene.
Different letters in the same row differ at P<0.05.

4. Discussion

The inclusion of AXT, LUT, and LYC, alone or in combination, did not affect (P>0.05) the zootechnical performance of laying hens, corroborating previous studies using low to moderate levels of AXT and LUT (Leeson and Caston, 2004; Yang et al., 2006; Leeson et al., 2007). In contrast, improvements in performance have been reported at higher inclusion levels of AXT and LYC, suggesting that the lack of response in the present study may be related to the dietary levels evaluated (Garcia et al., 2015; Magnuson et al., 2018).

The absence of improvements in the performance of laying hens supplemented with carotenoids in the present study may be associated with the inclusion levels evaluated, since positive responses have been reported in studies using higher supplementation levels than those tested here.

Most egg quality traits were unaffected by dietary treatments, except for yolk color, which was enhanced by LYC and by carotenoid combinations. These results agree with previous findings showing increased yolk pigmentation without effects on egg weight or internal quality parameters (Yang et al., 2006; Olson et al., 2008; Garcia et al., 2015). Leeson et al. (2007) observed no differences in egg and yolk weights with LUT, but reported increased yolk color with 125 mg kg^{-1} LUT supplementation. Magnuson et al. (2018) reported that dietary supplementation with AXT at 40 mg kg^{-1} significantly enhanced egg yolk color, which is consistent with the results observed in the present study for LYC. As hens do not synthesize carotenoids endogenously, yolk pigmentation is directly influenced by dietary carotenoid intake (Leeson and Caston, 2004), with corn-based diets already contributing lutein and zeaxanthin (Amaya et al., 2014).

No effects were observed on shell and external egg quality traits, consistent with previous studies (Leeson et al., 2007; Spada et al., 2012), although thicker shells have been reported with 40 mg kg^{-1} of AXT supplementation (Magnuson et al., 2018). Under room temperature storage, carotenoid supplementation did not affect internal egg quality; however, higher yolk MDA levels were observed in LYC-supplemented diets, likely due to the greater susceptibility of LYC to oxidative degradation (Rao and Agarwal, 2000). Leeson et al. (2007) noted that eggshell quality tended to decline towards the end of the laying period, posing challenges in demonstrating the positive effects of carotenoids on shell characteristics. Carotenoids did not affect external quality, likely due to their incorporation into chylomicrons and subsequent transport through the bloodstream (Parker, 1996).

For eggs stored at RT (25 °C), carotenoid supplementation did not affect characteristics like egg weight, HU, yolk index, or the pH of the albumen and yolk. However, MDA concentration in the yolk was higher in LYC-supplemented diets compared with the control diet and AXT-supplemented diets. This can be attributed to the greater susceptibility of LYC to oxidative degradation and isomerization (Rao and Agarwal, 2000).

Egg yolks from hens fed combinations of two carotenoids (AXT + LUT, AXT + LYC, or LUT + LYC) showed greater pigmentation than those receiving the triple combination, likely due to competition among carotenoids during intestinal uptake and metabolism, which can limit absorption and deposition in the yolk (Kostic et al., 1995). This competitive interaction can prevent the absorption and transport of carotenoids to target tissues (Salter-Venzon et al., 2017), potentially explaining the differences observed in yolk color.

Results showed varying MDA concentrations in egg yolks stored at RT, with AXT exhibiting superior antioxidant activity compared with other substances (Palozza et al., 2008). The instability of LYC resulted in higher MDA values in yolks, influenced by its chemical properties and susceptibility to oxidation or isomerization (Shi et al., 2002). Xanthophylls, such as LUT, are incorporated into the membrane with their terminal groups anchored in polar regions. In turn, LYC, due to the absence of hydrophilic substituents, remains entirely within the membrane. Therefore, carotenoids' specific structural characteristics may contribute to distinct protection mechanisms that achieve synergistic properties. The triple combination's inability to enhance yolk color may be attributed to carotenoid competition during absorption, leading to reduced deposition in the intestinal tract and subsequent intestinal absorption (Maiani et al., 2009).

Regarding individual carotenoids, LYC at 420 mg kg^{-1} efficiently improved yolk pigmentation by 4 mg kg^{-1} (Olson et al., 2008), and LUT at 500 mg kg^{-1} resulted in 2.2 mg of LUT in eggs (60 g), with increased yolk pigmentation (Leeson and Caston, 2004). Egg quality declined throughout RT storage, as evidenced by the reduction in egg weight caused by water loss from the albumen (Tainika et al., 2024).

Values of HU and YI decreased with storage due to carbonic acid (H_2CO_3) dissociation, breakdown of the ovomucin bonds, and increased pH in the yolk and albumen (Li-Chan et al., 1995). The RYC intensity decreased with storage, attributed to the rapid transfer of iron from the yolk to the egg white during storage, as well as to protein penetration into the yolk (Santos et al., 2009).

Under RF (8 °C), diets supplemented with LUT + LYC and AXT + LUT + LYC resulted in higher MDA concentrations than the CD and the AXT-supplemented diet. Yolk color increased with AXT + LUT, AXT + LYC, and LUT + LYC compared with AXT + LUT + LYC. Lower MDA concentrations in AXT-supplemented yolks were linked to the waterproofing effect of the unique chemical structure of AXT, which limits lipid peroxidation (Goto et al., 2001).

Carotenoid antioxidant activity is dose-dependent, and at lower concentrations these compounds may exhibit pro-oxidant effects. In this context, Garcia et al. (2015) demonstrated that dietary supplementation with 400 and 800 mg kg^{-1} of LYC in laying-hen diets significantly reduced MDA concentrations in eggs, indicating improved oxidative stability. Similarly, using seaweed as a dietary source of AXT, Magnuson et al. (2018) reported dose-dependent increases in AXT levels in plasma and liver of laying hens fed diets containing 10 and 80 mg kg^{-1}, demonstrating the hens' capacity to absorb, transport, and store algae-derived phytochemicals.

Phytochemicals are transported to different tissues, particularly to the liver where they can be stored or transported to other tissues. AXT may not be metabolized by animals, unlike most carotenoids, which indicates a unique retention of this compound in tissues and may explain the lower concentration of this carotenoid in egg yolks (Magnuson et al., 2018). Carotenoids' instability and susceptibility to oxidation contribute to yolk color loss during storage, a process that is accelerated by light, temperature, and the presence of metallic catalysts (Sarantópoulos et al., 2001).

The egg yolks produced by the hens supplemented with the combinations of AXT and LUT, AXT and LYC, and LUT and LYC showed greater pigmentation than those from hens fed the CD, LUT supplementation alone, or the combination of AXT + LUT + LYC. This result can be explained by the fact that hens primarily absorb substances present in LUT, known as oxycarotenoids, contained in the diets, especially in corn grains (Hudon, 1994).

In laying hens, oxycarotenoids stored in muscle and skin tissues are mobilized to the ovaries upon the onset of sexual maturity, and a portion of these compounds is subsequently deposited in the egg yolk, thereby determining yolk coloration (Goodwin, 1986). In general, animals are capable of absorbing, metabolizing, and depositing dietary carotenoids in various tissues, including the skin, beak, feet, and egg yolk (Breithaupt, 2007).

It has been shown that dietary supplementation with LUT at 250 mg kg^{-1} significantly intensified egg yolk color compared with a control diet without carotenoid inclusion, highlighting the strong pigmentation potential of this compound (Englmaierová and Skrivan, 2013). Similarly, Magnuson et al. (2018) reported increased egg yolk pigmentation in laying hens receiving AXT supplementation.

As expected, egg quality worsened during storage under RF. However, the loss of quality occurred less markedly in eggs stored under refrigeration (10 to 15 °C), according to Lana (2000). Egg weight (EW) and HU decreased, while the pH of the yolk and albumen increased with extended storage periods. These results were expected, as studies evaluating the effects of the tropical climate on eggs stored under RF have demonstrated that HU values decrease markedly during refrigerated storage, whereas yolk and albumen pH values increase due to the loss of carbon dioxide through the pores of the shell (Xavier et al., 2008). Jones et al. (2002) stated that storage time and temperature are important factors and must be controlled during storage. Thus, higher temperatures and longer storage times lead to a decline in internal egg quality, mainly indicated by lower HU values.

This fact accelerates the physicochemical reactions causing the degradation of the protein structure present in the albumin, resulting in the release of water previously bound to large protein molecules, which subsequently migrates to the yolk by osmosis (Leandro et al., 2005). The increase in yolk pH with longer storage periods aligns with findings by Shang et al. (2004). Similarly, the increase in albumen pH observed after 30 days of storage is associated with carbon dioxide loss through the enlarged pores of the shell (Li-Chan et al., 1995).

Yolk indexes obtained for eggs from laying hens that received the CD and those supplemented with AXT + LUT and AXT + LYC decreased after 15 days of storage. The progressive decrease in YI values during storage is explained by the gradual weakening of the vitelline membrane and the liquefaction of the yolk, which is mainly caused by the diffusion of water from the albumen (Akyurek and Okur, 2009).

The YI is based on the relationship between yolk height and diameter; thus, increased storage temperature may negatively affect this parameter, as observed in the present experiment. Eggs stored for up to six days after laying maintain high quality when kept at RT (25 °C), whereas eggs stored under RF (8 °C) maintain excellent quality for up to 30 days (Lana et al., 2018). Therefore, although yolk indexes decrease as storage periods increase, eggs maintained under RF tend to exhibit more stable YI values.

According to Santos et al. (2009), YI values should range between 0.35 and 0.49, indicating that all eggs analyzed when fresh and after 15 and 30 days of storage were within the range considered indicative of good quality. This variable has been recommended as one of the best indicators of egg quality when compared with HU and pH (Spada et al., 2012). Research on the inclusion of two antioxidants in the diets of laying hens is scarce. However, in the present study, the association of AXT and LUT, as well as the association of AXT and LYC, showed greater stabilization of YI values during storage.

5. Conclusions

Dietary supplementation with astaxanthin (AXT; 10 mg kg^{-1}), lutein (LUT; 10 mg kg^{-1}), and lycopene (LYC; 10 mg kg^{-1}), either individually or in combination, did not influence the zootechnical performance or overall egg quality of laying hens. Dietary supplementation with AXT improves the oxidative stability of eggs compared with LYC, suggesting a greater protective effect of this carotenoid against oxidative deterioration. Combined supplementation with AXT + LUT, AXT + LYC, and LUT + LYC enhances yolk pigmentation, whereas the simultaneous inclusion of all three carotenoids reduces pigmentation intensity. Regardless of dietary treatment, egg storage for up to 30 days, both at room temperature

(25 °C) and under refrigeration (8 °C), negatively affects the internal quality of eggs. These findings highlight the potential of specific carotenoid combinations to improve yolk pigmentation and oxidative stability, while also reinforcing the importance of appropriate storage conditions to preserve egg quality.

Data availability

All data generated or analyzed during this study are included in this published article.

Author contributions

Conceptualization: Santana, P. G.; Garcia, E. R. M. and Melo, E. P. **Data curation:** Ferraz, A. L. J. **Investigation:** Santana, P. G.; Garcia, E. R. M.; Andrade, G. C. and Souza, A. F. G. O. **Methodology:** Garcia, E. R. M.; Andrade, G. C.; Ferraz, A. L. J.; Souza, A. F. G. O.; Melo, E. P. and Kiefer, C. **Project administration:** Garcia, E. R. M. **Supervision:** Garcia, E. R. M. **Writing – original draft:** Santana, P. G.; Garcia, E. R. M.; Andrade, G. C.; Ferraz, A. L. J.; Souza, A. F. G. O.; Melo, E. P. and Kiefer, C. **Writing – review & editing:** Garcia, E. R. M. and Kiefer, C.

Conflict of interest

The authors declare no conflict of interest.

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References

Akyurek, H. and Okur, A. A. 2009. Effect of storage time, temperature and hen age on egg quality in free-range layer hens. Journal of Animal and Veterinary Advances 8:1953-1958.

Amaya, E.; Becquet, P.; Carné, S.; Peris, S. and Miralles, P. 2014. Carotenoids in animal nutrition. Fefana, Brussels.

Breithaupt, D. E. 2007. Modern application of xanthophylls in animal feeding: a review. Trends in Food Science and Technology 18:501-506. https://doi.org/10.1016/j.tifs.2007.04.009

Carvalho, F. B.; Stringhini, J. H.; Jardim Filho, R. M.; Leandro, N. S. M.; Café, M. B. and Deus, H. A. S. B. 2007. Qualidade interna e da casca para ovos de poedeiras comerciais de diferentes linhagens e idade. Ciência Animal Brasileira 8:25-29.

Dekalb White. 2009. Manual de manejo das poedeiras Dekalb White. Modelo de Revisão. 39p.

Englmaierová, M. and Skrivan, M. 2013. Effect of synthetic carotenoids, lutein, and mustard on the performance and egg quality. Scientia Agriculturae Bohemica 44:138-143.

Darvin, M. E.; Lademann, J.; von Hagen, J.; Lohan, S. B.; Kolmar, H.; Meinke, M. C. and Jung, S. 2022. Carotenoids in human skin in vivo: Antioxidant and photo-protectant role against external and internal stressors. Antioxidants 11:1451. https://doi.org/10.3390/antiox11081451

Fontana, J. D.; Mendes, S. V.; Persike, D. S.; Peracetta, L. and Passos, M. 2000. Carotenóides cores atraentes e ação biológica. Biotecnologia Ciência & Desenvolvimento 13:40-45.

Garcia, E. R. M.; Cruz, F. K.; Kiefer, C.; Avila, L. R. and Souza, R. P. P. 2015. Minerais orgânicos e licopeno na alimentação de poedeiras: desempenho zootécnico e qualidade dos ovos. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 67:1703-1710. https://doi.org/10.1590/1678-4162-8418

Goodwin, T. W. 1986. Metabolism, nutrition and function of carotenoids. Annual Review of Nutrition 6:273-297. https://doi.org/10.1146/annurev.nu.06.070186.001421

Goto, S.; Kogure, K.; Abe, K.; Kimata, Y.; Kitahama, K.; Yamashita, E. and Terada, H. 2001. Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin. Biochimica et Biophysica Acta (BBA) - Biomembranes 1512:251-258. https://doi.org/10.1016/S0005-2736(01)00326-1

Hamilton, R. M. G. 1982. Methods and factors that affect the measurement of egg shell quality. Poultry Science 61:2022-2039. https://doi.org/10.3382/ps.0612022

Hayat, Z.; Cherian, G.; Pasha, T. N.; Khattak, F. M. and Jabbar, M. A. 2010. Oxidative stability and lipid components of eggs from flax-fed hens: Effect of dietary antioxidants and storage. Poultry Science 89:1285-1292. https://doi.org/10.3382/ps.2009-00256

Hudon, J. 1994. Biotechnological applications of research on animal pigmentation. Biotechnology Advances 12:49-69. https://doi.org/10.1016/0734-9750(94)90290-9

Jardini, F. A. and Mancini Filho, J. 2007. Avaliação da atividade antioxidante em diferentes extratos da polpa e sementes da romã (*Punica granatum*, L.). Revista Brasileira de Ciências Farmacêuticas 43:137-147. https://doi.org/10.1590/S1516-93322007000100017

Jones, D. R.; Tharrington, J. B.; Curtis, P. A.; Anderson, K. E.; Keener, K. M. and Jones, F. T. 2002. Effects of cryogenic cooling of shell eggs on egg quality. Poultry Science 81:727-733. https://doi.org/10.1093/ps/81.5.727

Kostic, D.; White, W. S. and Olsen, J. A. 1995. Intestinal absorption, serum clearance, and interactions between lutein and B-carotene when administered to human adults in separate or combined oral doses. American Journal of Clinical Nutrition 62:604-610. https://doi.org/10.1093/ajcn/62.3.604

Lana, G. R. Q. 2000. Avicultura. Livraria e Editora Rural Ltda., Campinas.

Lana, S. R. V.; Lana, G. R. Q.; Silva, L. C. L.; Salvador, E. L.; Leão, A. P. A.; Lana, A. M. Q. and Barros Jr, R. F. 2018. Effect of temperature and storage time on the quality of eggs from commercial laying hen. Archivos de Zootecnia 67:93-98.

Leandro, N. S. M.; Deus, H. A. B.; Stringhini, J. H.; Café, M. B.; Andrade, M. A. and Carvalho, F. B. 2005. Aspectos de qualidade interna e externa de ovos comercializados em diferentes estabelecimentos na região de Goiânia. Ciência Animal Brasileira 6:71-78.

Leeson, S. and Caston, L. 2004. Enrichment of eggs with lutein. Poultry Science 83:1709-1712. https://doi.org/10.1093/ps/83.10.1709

Leeson, S.; Caston, L. and Namkung, H. 2007. Effect of dietary lutein and flax on performance, egg composition and liver status of laying hens. Canadian Journal of Animal Science 87:365-372. https://doi.org/10.4141/A06-043

Li-Chan, E.; Powrie, W. D. and Nakai, S. 1995. The chemistry of eggs and egg products. p.109-160. In: Egg science and technology. 4th ed. Stadelman, W. J. and Cotterill, O. J., eds. Food Products Press, New York.

Maiani, G.; Periago Castón, M. J.; Catasta, G.; Toti, E.; Cambrodón, I. G.; Bysted, A.; Granado-Lorencio, F.; Olmedilla-Alonso, B.; Knuthsen, P.; Valoti, M.; Böhm, V.; Mayer-Miebach, E.; Behsnilian, D. and Schlemmer, U. 2009. Carotenoids: Actual knowledge of food sources, intakes, stability and bioavailability and their protective role in humans. Molecular Nutrition & Food Research 53:S194-S218. https://doi.org/10.1002/mnfr.200800053

Magnuson, A. D.; Sun, T.; Yin, R.; Liu, G.; Tolba, S.; Shinde. S. and Lei, X. G. 2018. Supplemental microalgal astaxanthin produced coordinated changes in intrinsic antioxidant systems of layer hens exposed to heat stress. Algal Research 33:84-90. https://doi.org/10.1016/j.algal.2018.04.031

Nabi, F.; Arain, M. A.; Rajput, N.; Alagawany, M.; Soomro, J.; Umer, M.; Soomro, F.; Wang, Z.; Ye, R. and Liu, J. 2020. Health benefits of carotenoids and potential application in poultry industry: A review. Journal of Animal Physiology and Animal Nutrition 104:1809-1818. https://doi.org/10.1111/jpn.13375

Olson, J. B.; Ward, N. E. and Koutsos, E. A. 2008. Lycopene incorporation into egg yolk and effects on laying hen immune function. Poultry Science 87:2573-2580. https://doi.org/10.3382/ps.2008-00072

Palozza, P.; Barone, E.; Mancuso, C. and Picci, N. 2008. The protective role of carotenoids against 7-keto-cholesterol formation in solution. Molecular and Cellular Biochemistry 309:61-68. https://doi.org/10.1007/s11010-007-9643-y

Parker, R. S. 1996. Absorption, metabolism, and transport of carotenoids. The FASEB Journal 10:542-551. https://doi.org/10.1096/fasebj.10.5.8621054

Panaite, T. D.; Nour, V.; Saracila, M.; Turcu, R. P.; Untea, A. E. and Vlaicu, P. A. 2021. Effects of linseed meal and carotenoids from different sources on egg characteristics, yolk fatty acid and carotenoid profile and lipid peroxidation. Foods 10:1246. https://doi.org/10.3390/foods10061246

Pita, M. C. G.; Piber Neto, E.; Nakaoka, L. M. and Mendonça Junior, C. X. 2004. Efeito da adição de ácidos graxos insaturados e de vitamina E à dieta de galinhas e seu reflexo na composição lipídica e incorporação de α-tocoferol na gema do ovo. Brazilian Journal of Veterinary Research and Animal Science 41:25-31.

Puglisi, M. J. and Fernandez, M. L. 2022. The health benefits of egg protein. Nutrients 14:2904. https://doi.org/10.3390/nu14142904

Rafed, R.; Abedi, M. H. and Mushfiq, S. R. 2024. Nutritional value of eggs in human diet. Journal for Research in Applied Sciences and Biotechnology 3:172-176. https://doi.org/10.55544/jrasb.3.1.28

Ramanatham, L. and Das, N. P. 1992. Studies on the control of lipid oxidation in ground fish by some polyphenolic natural products. Journal of Agricultural and Food Chemistry 40:17-21. https://doi.org/10.1021/JF00013A004

Rao, A. V. and Agarwal, S. 2000. Role of antioxidant lycopene in cancer and heart disease. Journal of the American College of Nutrition 19:563-569. https://doi.org/10.1080/07315724.2000.10718953

Rostagno, H. S.; Albino, L. F. T.; Hannas, M. I.; Donzele, J. L.; Sakomura, N. K.; Perazzo, F. G.; Saraiva, A.; Teixeira, M. L.; Rodrigues, P. B.; Oliveira, R. F.; Barreto, S. L. T. and Brito, C. O. 2017. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. UFV, Departamento de Zootecnia, Viçosa, MG.

Salter-Venzon, D.; Kazlova, V.; Izzy Ford, S.; Intra, J.; Klosner, A. E. and Gellenbeck, K. W. 2017. Evidence for decreased interaction and improved carotenoid bioavailability by sequential delivery of a supplement. Food Science & Nutrition 5:424-433. https://doi.org/10.1002/fsn3.409

Santos, M. S. V.; Espíndola, G. B.; Lôbo, R. N. B.; Freitas, E. R.; Guerra, J. L. L. and Santos, A. B. E. 2009. Efeito da temperatura e estocagem em ovos. Food Science and Technology 29:513-517. https://doi.org/10.1590/S0101-20612009000300009

Sarantópoulos, C. I. G. L.; Oliveira, L. M. and Canavesi, E. 2001. Requisitos de conservação de alimentos em embalagens flexíveis. CETEA/ITAL, Campinas.

Shang, X. G.; Wang, F. L.; Li, D. F.; Yin, D. J. and Li, J. Y. 2004. Effects of dietary conjugated linoleic acid on the productivity of laying hens and egg quality during refrigerated storage. Poultry Science 83:1688-1695. https://doi.org/10.1093/ps/83.10.1688

Shi, J.; Le Maguer, M. and Bryan, M. 2002. Lycopene from tomatoes. p.135-163. In: Shi, J.; Le Maguer, M. and Mazza, G. (eds.). Functional foods: Biochemical and processing aspects. v.2. Functional Foods and Nutraceuticals Series. CRC Press.

Silversides, F. G. and Budgell, K. 2004. The relationships among measures of egg albumen height, pH, and whipping volume. Poultry Science 83:1619-1623. https://doi.org/10.1093/ps/83.10.1619

Spada, F. P.; Brazaca, S. G. C.; Coelho, A. D.; Savino, V. J. M.; França, L. C.; Correr, E.; Martins, E.; Fisher, F. S. and Lemes, D. E. A. 2012. Adição de carotenóides naturais e artificiais na alimentação de galinhas poedeiras: efeitos na qualidade de ovos frescos e armazenados. Ciência Rural 42:346-353. https://doi.org/10.1590/S0103-84782012000200025

Tainika, B.; Abdallah, N.; Damaziak, K.; Waithaka Ng'ang'a, Z.; Shah, T. and Wójcik, W. 2024. Egg storage conditions and manipulations during storage: effect on egg quality traits, embryonic development, hatchability and chick quality of broiler hatching eggs. World's Poultry Science Journal 80:75-107. https://doi.org/10.1080/00439339.2023.2252785

Vyncke, B. W. 1970. Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. Fette, Seifen, Anstrichmittel 72:1084-1087. https://doi.org/10.1002/lipi.19700721218

Xavier, I. M. C.; Cançado, S. V.; Figueiredo, T. C.; Lara, L. J. C.; Lana, A. M. Q.; Souza, M. R. and Baião, N. C. 2008. Qualidade de ovos de consumo submetidos a diferentes condições de armazenamento. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 60:953-959. https://doi.org/10.1590/S0102-09352008000400026

Yang, Y. X.; Kim, Y. J.; Jin, Z.; Lohakare, J. D.; Kim, C. H.; Ohh, S. H.; Lee, S. H.; Choi, J. Y. and Chae, B. J. 2006. Effects of dietary supplementation of astaxanthin on production performance, egg quality in layers and meat quality in finishing pigs. Asian-Australasian Journal of Animal Science 19:1019-1025. https://doi.org/10.5713/ajas.2006.1019

Zaheer, K. 2015. An updated review on chicken eggs: production, consumption, management aspects and nutritional benefits to human health. Food and Nutrition Sciences 6:1208-1220. https://doi.org/10.4236/fns.2015.613127