Effects of supplementing natural astaxanthin from *Haematococcus pluvialis* to laying hens on egg quality during storage at 4°C and 25°C

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ABSTRACT The objective of this study was to evaluate the effects of different levels of dietary natural astaxanthin (**ASTA**) (from the microalga *Haematococcus pluvialis*) and storage at 4°C and 25°C on the quality of eggs from laying hens. Nongda No. 3 laying hens (n = 450) were randomly allocated to 1 of 5 dietary treatments. Each treatment had 6 replicates of 15 hens each. All birds were assigned to a corn–soybean meal–based diet containing 0, 20, 40, 80, or 160 mg/kg natural ASTA for 4 wk. A total of 540 eggs were collected at the end of the 4-week feeding trial. Sixty fresh eggs were collected and measured for egg quality within 24 h after collection. The other 480 eggs were used in a factorial arrangement with 5 dietary ASTA levels, 4 storage times, and 2 storage temperatures. During the 8-week storage period at 4°C and 25°C, egg quality measurements were performed every 2 wk on 12 eggs per treatment. No significant effects ($P > 0.05$) on yolk index, yolk pH, Haugh units, weight loss, or eggshell strength were observed with increasing concentrations of dietary ASTA. Yolk color darkened linearly with increasing dose of ASTA ($P < 0.05$). During storage of eggs, yolk index and Haugh units decreased significantly ($P < 0.05$), whereas yolk pH and weight loss increased ($P < 0.05$). An interaction was observed between dietary ASTA level and storage time on yolk index, yolk color, and Haugh units ($P < 0.05$). These results demonstrated that dietary ASTA from *H. pluvialis* delayed the decrease in yolk index and yolk color during storage at 4°C and 25°C. Therefore, we speculate that there may be a combined effect of dietary ASTA level and storage time on egg internal quality; this information may provide additional options by which to extend the storage time of eggs.

Key words: natural astaxanthin, chicken egg, storage temperature, egg quality

2020 Poultry Science 99:6877–6883
https://doi.org/10.1016/j.psj.2020.09.010

INTRODUCTION

Natural astaxanthin (**ASTA**), an oxygenated derivative of carotenoid, has various beneficial characteristics, such as improving antioxidant capacity (Zhao et al., 2019) and inhibiting lipid peroxidation (Naguib, 2000), as well as antiaging effects (Nootem et al., 2018) among others. Astaxanthin is one of the strongest antioxidants found in nature (Jingyao et al., 2017); it has a 3S,3′S configuration that makes it more stable and resistant to oxidation than synthetic ASTA (Starr, 1976; Bikadi et al., 2006). Dietary levels of ASTA from algae have been shown to darken egg yolk in a dose-dependent manner and to improve antioxidant capacity in laying hens (Walker et al., 2012).

The microalga *Haematococcus pluvialis* is one of the most effective organisms for production of ASTA; it can produce a large amount of ASTA under certain conditions (Sarada et al., 2002; Shao et al., 2019). Supplementation of ASTA (from *H. pluvialis*) to the diet can improve egg yolk color, increase total antioxidant capacity, and inhibit lipid peroxidation (Yang et al., 2011; Li et al., 2018). A previous study showed that feeding garlic to laying hens increased antioxidant enzyme activity and storage time of eggs by increasing the antioxidant capacity (Mahmoud et al., 2010). However, the effect of interactions between dietary ASTA levels and storage time on the quality of eggs remains unclear. Progressive deterioration of egg quality during egg storage has been shown to be related to the management and feeding of laying hens (Vits et al., 2005; Adabi et al., 2010). Factors

Received June 1, 2020.
Accepted September 7, 2020.

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associated with egg handling and storage temperature also affect egg quality during storage (Merritt, 1955). A previous study showed that an interaction between storage temperature and time affected the quality of eggs (Han et al., 2011). Because ASTA is good for hens' health and can be readily transferred from the diet to the yolk, we speculated that the storage time of eggs from hens fed ASTA could be extended without loss of egg quality.

The objective of this study was to comprehensively investigate the effects of dietary supplementation with ASTA (from *H. pluvialis*) and storage time on yolk index, yolk pH, yolk color, weight loss, and Haugh units (**HU**) of eggs stored at 4°C and 25°C.

MATERIALS AND METHODS

Experimental Materials

H. pluvialis was purchased from Jingzhou Natural Astaxanthin Ltd. (Hubei, China), and its ASTA content was 1.5%.

Experimental Birds and Dietary Treatments

All experimental protocols were approved by the Animal Care and Use Committee of Beijing University of Agriculture (Beijing, China). In total, 450 Nongda No. 3 laying hens, 350 d of age, were randomly allotted to 5 treatment groups that varied in dietary ASTA concentration: 0, 20, 40, 80, or 160 mg/kg of ASTA. Each treatment had 6 replicates of 15 birds, with 3 birds per cage. All birds were fed a basal diet for 1 wk and then assigned to a corn–soybean meal–based diet containing 0, 20, 40, 80, or 160 mg/kg of ASTA for 4 wk. At the end of the 4-week feeding trial, 540 eggs were collected for assessment of egg quality. The eggs had no defects (cracks or breaks), and egg weights were close to the average egg weight for each replicate. The ingredients and nutritional composition of the basal diet are shown in Table 1.

Table 1. The composition and nutritional level of the basal diet (air-dried basis) fed to laying hens.

Ingredients	Content[%]	Nutrient level	Content[%]
Corn	63.30	ME [MJ/kg]	10.96
Soybean meal	23.75	CP [%]	16.10
Cottonseed meal	1.00	DL-Methionine [%]	0.368
DL-Methionine	0.10	L-Lysine [%]	0.750
Limestone	8.70	Total calcium [%]	3.51
$CaHPO_4$	1.80	Total phosphorus [%]	0.62
NaCl	0.35	Available phosphorus [%]	0.44
Premix[1]	1.00		
Total	100.00		

[1]Premix provided per kg of diet: vitamin A, 13,000 IU; vitamin D_3, 6,000 IU; vitamin E, 20 IU; vitamin K, 2 mg; vitamin B_1, 1 mg; vitamin B_2, 9 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.006 mg; folic acid, 0.3 mg; calcium pantothenate, 6 mg; niacin, 20 mg; biotin, 0.2 mg; Cu, 10.04 mg; Fe, 60 mg; Mn, 95.4 mg; Zn, 103.5 mg; I, 0.4 mg; Se, 0.3 mg.

Experimental Design and Storage of Eggs

Sixty fresh eggs were collected and measured for egg quality within 24 h after collection. The other 480 eggs were used in a factorial arrangement with 5 dietary ASTA levels × 5 storage times × 2 storage temperature conditions. All of the eggs were placed with small end down (Su et al., 2009) on egg racks and stored at 4°C or 25°C for 8 wk. During the 8-wk period, egg quality measurements were performed every 2 wk on 12 eggs per temperature treatment.

Egg Quality Measurements

Haugh units and yolk color of each egg were measured using an egg analyzer (Orka Food Technology Ltd., Ramat Hasharon, Israel) (Wang et al., 2015a,b). Yolk color was defined as per the Roche yolk color fan, where 1 represents bright yellow and 15 represents dark yellow (XiaoLong et al., 2011). Weight loss was calculated as follows: weight loss = ([primary whole egg weight at day 0 − whole egg weight after storage]/primary whole egg weight at day 0) × 100. An egg force reader (Orka Food Technology Ltd.) was used to measure the eggshell strength of eggs (Wang et al., 2015a,b). Yolk pH was measured by using a pH meter (pH Spear; Eutech Instruments, Vernon Hills, IL) immediately after the egg white and yolk were completely separated (Han et al., 2011).

Determination of ASTA in Egg Yolk

The egg yolk was freeze-dried using a lyophilizer (NAI; Shanghai, China). The egg yolk was ground, placed in a bag, which was sealed and stored in a refrigerator at −80°C. Then, 2.5 g of lyophilized yolk sample and 5 mL of deionized water were added to a 50-mL centrifuge tube that was placed in a 50°C ultrasonic machine for 30 min after cooling. The yolk sample in the centrifuge tube was washed 3 times with 30 mL of dichloromethane. The contents of the centrifuge tube were placed in a separatory funnel and filtered 3 times. Chromatographic separation was performed using an Alliance 2695 HPLC instrument (Waters, Milford, MA). The analysis time was 10 min, which was followed by a re-equilibration time of 2 min, for a total run time of 12 min. The flow rate of the mobile phase was 1 mL/min, the injection volume was 10 μL, the column temperature was maintained at 30°C, and ASTA was detected at a wavelength of 474 nm.

Statistical Analysis

All data were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY). Data related to the effect of interaction between dietary ASTA level and storage time on egg quality were analyzed using the GLM procedure as a 5 × 5 factorial arrangement, with diet (ASTA level) and storage time as the main effects. Data related to the effect of dietary ASTA level on egg quality and effect

Table 2. Effect of dietary astaxanthin and storage time on quality of eggs stored at 4°C.[1]

Storage time	Natural astaxanthin (mg/kg)	Yolk quality parameter			Weight loss(%)	Haugh unit	Eggshell strength (N/cm^2)
		Yolk index	Yolk color	Yolk pH			
0 wk	0	0.52^{a}	10.71^{h,i}	6.01	0.00	83.40^{a}	41.92
	20	0.51^{a,b,c}	11.68^{g,h}	6.00	0.00	83.43^{a}	40.23
	40	0.51^{a,b,c}	12.84^{e,f,g}	5.99	0.00	82.88^{a,b}	41.92
	80	0.51^{a,b,c}	14.47^{a,b,c}	6.00	0.00	82.25^{a,b,c}	42.45
	160	0.51^{a,b,c}	14.89^{a,b}	6.00	0.00	83.34^{a}	43.17
2 wk	0	0.43^{g,h}	9.50^{i,j}	6.22	3.12	82.03^{a,b,c}	40.90
	20	0.43^{g,h}	12.00^{f,g,h}	6.18	3.23	80.67^{a,b,c,d}	39.16
	40	0.43^{g,h}	13.17^{c,d,e,f}	6.25	2.95	81.98^{a,b,c}	41.47
	80	0.42^{h}	14.50^{a,b}	6.23	3.26	78.55^{a,b,c,d,e}	37.76
	160	0.44^{e,f,g,h}	15.00^{a}	6.18	3.19	83.25^{a,b}	41.91
4 wk	0	0.44^{f,g,h}	9.17^{j}	6.38	1.35	81.77^{a,b,c}	41.17
	20	0.44^{f,g,h}	12.67^{e,f,g}	6.31	1.49	79.68^{a,b,c,d,e}	41.97
	40	0.43^{g,h}	13.67^{b,c,d,e}	6.25	1.48	77.52^{a,b,c,d,e}	40.56
	80	0.44^{f,g,h}	14.67^{a,b}	6.38	1.45	77.78^{a,b,c,d,e}	38.81
	160	0.44^{f,g,h}	15.00^{a}	6.36	1.44	75.98^{a,b,c,d,e}	41.94
6 wk	0	0.47^{b,c,d,e,f,g}	9.17^{j}	6.49	2.32	72.13^{d,e}	41.11
	20	0.48^{a,b,c,d,e,f}	13.00^{d,e,f}	6.49	2.18	73.25^{c,d,e}	38.73
	40	0.50^{a,b,c,d}	13.67^{b,c,d,e}	6.56	2.18	78.68^{a,b,c,d,e}	42.57
	80	0.51^{a,b,c}	14.67^{a,b}	6.55	2.27	76.85^{a,b,c,d,e}	40.42
	160	0.51^{a,b}	15.00^{a}	6.39	2.26	82.00^{a,b,c}	40.71
8 wk	0	0.45^{d,e,f,g,h}	9.50^{i,j}	6.32	2.49	71.20^{e}	40.22
	20	0.46^{c,d,e,f,g,h}	12.50^{e,f,g}	6.44	2.59	74.52^{a,b,c,d,e}	41.54
	40	0.47^{b,c,d,e,f,g}	14.17^{a,b,c,d}	6.38	2.56	74.08^{b,c,d,e}	42.36
	80	0.49^{a,b,c,d,e}	15.00^{a}	6.27	2.46	77.22^{a,b,c,d,e}	43.44
	160	0.51^{a,b,c}	15.00^{a}	6.47	2.44	80.47^{a,b,c,d}	41.58
Pooled SEM		0.003	0.168	0.017	0.090	0.442	0.299
Source of variation							
P-value	Dietary natural astaxanthin levels	0.023	<0.001	1.000	0.586	0.065	0.386
	Storage time	<0.001	0.100	<0.001	<0.001	<0.001	0.320
	Dietary natural astaxanthin levels × time	0.032	<0.001	0.146	0.237	0.003	0.743

[a–j]Mean values within a column without common superscripts differ significantly ($P < 0.05$).
[1]Data were analyzed by GLM as a 5 × 5 factorial arrangement of dietary ASTA level and storage time as the main effects. SEM = standard error of mean values (n = 150).

of storage time on egg quality were analyzed by 1-way ANOVA with orthogonal linear and quadratic contrasts. Tukey's multiple comparison test was used to examine statistical differences among treatments. Statistical significance was defined at $P < 0.05$.

RESULTS

Yolk Index, Yolk Color, and pH of Raw Eggs at 4°C

The effects of dietary ASTA levels and storage time on yolk index, yolk color, and yolk pH in raw eggs stored at 4°C are shown in Table 2. Dietary ASTA level did not affect yolk index or pH ($P > 0.05$; Table 3), but yolk color increased linearly ($P < 0.05$). Yolk index declined quadratically ($P < 0.05$; Table 4), and yolk pH increased linearly ($P < 0.05$) with storage time compared with the control group. We also found a significant interaction between dietary ASTA level and storage time on yolk index and yolk color ($P < 0.05$; Table 2). The egg yolk index in the 160 mg/kg ASTA group was significantly higher than that in the control group for eggs stored at 4°C for 8 wk ($P < 0.05$; Table 2).

Weight Loss, HU, and Eggshell Strength of Raw Eggs at 4°C

Dietary ASTA level did not affect weight loss, HU, or eggshell strength ($P > 0.05$; Table 3). As storage time increased, the weight loss of eggs increased linearly ($P < 0.05$; Table 4) and HU declined linearly at

Table 3. Effect of dietary natural astaxanthin level on quality of eggs stored at 4°C.[1]

Item	Natural astaxanthin (mg/kg)					SEM	*P*-value		
	0	20	40	80	160		ANOVA	Linear	Quadratic
Yolk index	0.46	0.46	0.47	0.47	0.48	0.003	0.457	0.080	0.496
Yolk color	9.61^{d}	12.37^{c}	13.50^{b}	14.66^{a}	14.98^{a}	0.168	<0.001	<0.001	<0.001
Yolk pH	6.28	6.29	6.29	6.28	6.28	0.017	1.000	0.958	0.925
Weight loss (%)	1.86	1.90	1.83	1.89	1.86	0.090	0.999	0.991	0.986
Haugh unit	78.11	78.31	79.03	78.53	81.01	0.442	0.226	0.054	0.366
Eggshell strength (N/cm2)	41.06	40.32	41.78	40.58	41.86	0.299	0.373	0.383	0.577

[a–d]Mean values within a row without common superscripts differ significantly ($P < 0.05$).
[1]Data were analyzed by 1-way ANOVA with orthogonal linear and quadratic contrasts.

Table 4. Effect of storage time on quality of eggs stored at 4°C.[1]

Item	Storage time (wk)					SEM	*P*-value		
	0	2	4	6	8		ANOVA	Linear	Quadratic
Yolk index	0.51[a]	0.43[c]	0.43[c]	0.49[a]	0.48[b]	0.003	<0.001	0.745	<0.001
Yolk color	12.92	12.83	13.03	13.10	13.23	0.168	0.954	0.456	0.834
Yolk pH	6.00[d]	6.21[c]	6.34[b]	6.50[a]	6.37[b]	0.017	<0.001	<0.001	<0.001
Weight loss (%)	0.00[e]	3.15[a]	1.44[d]	2.24[c]	2.51[b]	0.090	<0.001	<0.001	<0.001
Haugh unit	83.06[a]	81.30[a,b]	78.55[b,c]	76.58[c]	75.50[c]	0.442	<0.001	<0.001	0.504
Eggshell strength (N/cm2)	41.94	40.24	40.89	40.71	41.83	0.299	0.305	0.906	0.056

[a–e]Mean values within a row without common superscripts differ significantly ($P < 0.05$).
[1]Data were analyzed by 1-way ANOVA with orthogonal linear and quadratic contrasts.

4°C ($P < 0.05$). A significant interaction was noted between dietary ASTA level and storage time at 4°C on HU ($P < 0.05$). Haugh units were significantly higher in the 160 mg/kg ASTA group than in the control group of eggs stored at 4°C for 6 or 8 wk ($P < 0.05$; Table 2).

Yolk Index, Yolk Color, and pH of Raw Eggs at 25°C

The effects of dietary ASTA levels and storage time on yolk index, yolk color, and pH in raw eggs stored at 25°C are shown in Table 5. Yolk color darkened linearly with increasing dietary ASTA levels ($P < 0.05$; Table 6). Dietary ASTA level did not affect yolk index ($P > 0.05$). Storage time strongly affected yolk index and yolk pH of raw eggs stored at 25°C ($P < 0.05$; Table 7). The yolk index of eggs at week 0 was higher than that of stored eggs ($P < 0.05$). We found a significant interaction between dietary ASTA level and storage time on yolk index and yolk color in eggs stored at 25°C ($P < 0.05$; Table 5). Yolk index was significantly higher in the 80 and 160 mg/kg ASTA groups than in the control group in eggs stored at 25°C for 6 wk ($P < 0.05$; Table 5).

Weight Loss, HU, and Eggshell Strength of Raw Eggs at 25°C

Table 5 shows the effects of dietary ASTA level and storage time on weight loss, HU, and eggshell strength of raw eggs. Dietary ASTA level did not affect weight

Table 5. Effect of dietary natural astaxanthin and storage time on quality of eggs stored at 25°C[1].

Storage time	Natural astaxanthin (mg/kg)	Yolk quality parameter			Weight loss (%)	Haugh unit	Eggshell strength (N/cm^2)
		Yolk index	Yolk color	Yolk pH			
0 wk	0	0.52[a]	10.71[f]	6.01	0.00	83.40	41.92
	20	0.51[a]	11.68[e,f]	6.00	0.00	83.43	40.23
	40	0.51[a]	12.84[c,d]	5.99	0.00	82.88	41.92
	80	0.51[a]	14.47[a,b]	6.00	0.00	82.25	42.45
	160	0.51[a]	14.89[a]	6.00	0.00	83.34	43.17
2 wk	0	0.36[b]	9.17[g]	6.51	0.65	52.48	41.61
	20	0.38[b]	12.17[d,e]	6.32	0.65	59.08	42.59
	40	0.38[b]	13.67[b,c]	6.33	0.58	54.93	42.08
	80	0.37[b]	14.83[a]	6.30	0.62	52.38	41.71
	160	0.36[b]	15.00[a]	6.23	0.65	58.10	41.35
4 wk	0	0.25[c,d]	10.83[f]	6.55	6.36	34.87	41.54
	20	0.26[c]	13.00[c,d]	6.52	6.62	36.73	41.95
	40	0.24[c,d,e]	14.50[a,b]	6.48	6.61	33.68	43.39
	80	0.24[c,d,e]	14.83[a]	-*	6.89	40.07	42.13
	160	0.24[c,d,e]	15.00[a]	-*	6.72	37.05	40.48
6 wk	0	0.15[g,h]	-*	6.69	11.93	-*	42.10
	20	0.15[g,h]	-*	6.82	11.50	-*	42.74
	40	0.18[f,g]	-*	6.86	11.57	-*	41.95
	80	0.20[e,f]	-*	6.52	10.83	-*	41.62
	160	0.21[d,e,f]	-*	6.70	11.41	-*	42.74
8 wk	0	0.14[g,h]	-*	6.93	15.66	-*	42.56
	20	0.13[h]	-*	6.76	15.12	-*	41.80
	40	0.15[g,h]	-*	6.78	15.40	-*	42.05
	80	0.15[g,h]	-*	6.63	15.26	-*	43.52
	160	0.17[f,g,h]	-*	6.68	15.28	-*	41.88
Pooled SEM		0.011	0.205	0.030	0.508	2.145	0.288
Source of variation							
P-value	Dietary natural astaxanthin levels	0.081	<0.001	0.476	0.909	0.528	0.986
	Storage time	<0.001	<0.001	<0.001	<0.001	<0.001	0.981
	Dietary natural astaxanthin levels × time	<0.001	<0.001	0.443	0.944	0.536	0.996

[a–h]Mean values within a column without common superscripts differ significantly ($P < 0.05$).
*Not determined because the Haugh unit was very low (<25) and yolk breakage.
[1]Data were analyzed by GLM procedure as a 5 × 5 factorial arrangement with dietary ASTA level and storage time as the main effects. SEM = standard error of mean values (n = 150).

Table 6. Effect of dietary natural astaxanthin levels on quality of eggs stored at 25°C.[1]

	Natural astaxanthin (mg/kg)						*P*-value		
Item	0	20	40	80	160	SEM	ANOVA	Linear	Quadratic
Yolk index	0.28	0.28	0.29	0.29	0.30	0.011	0.990	0.606	0.990
Yolk color	10.24^{d}	12.28^{c}	13.56^{b}	14.71^{a}	15.96^{a}	0.205	<0.001	<0.001	<0.001
Yolk pH	6.54	6.48	6.49	-*	-*	0.038	0.813	0.607	0.709
Weight loss (%)	6.92	6.78	6.85	6.72	6.82	0.508	1.000	0.944	0.950
Haugh unit	56.92	59.75	60.10	58.23	62.30	2.145	0.953	0.522	0.989
Eggshell strength (N/cm2)	41.95	41.86	42.20	42.29	42.03	0.288	0.990	0.776	0.809

[a–d]Mean values within a row without common superscripts differ significantly ($P < 0.05$).
*Not determined because the Haugh unit was very low (<25) and yolk breakage.
[1]Data were analyzed by 1-way ANOVA with orthogonal linear and quadratic contrasts.

loss, HU, or eggshell strength of eggs at 25°C ($P > 0.05$; Table 6). Storage time linearly increased the weight loss of raw eggs and decreased HU at 25°C ($P < 0.05$; Table 7).

ASTA Concentration in Egg Yolk

Table 8 shows the effect of dietary ASTA levels on the concentrations of ASTA in egg yolk. The concentration of ASTA in egg yolk increased linearly with increasing dietary ASTA level ($P < 0.05$).

DISCUSSION

This study was designed to investigate the effects of levels of dietary ASTA (from *H. pluvialis*) and storage time on the quality of eggs. Except for yolk color, the internal quality of raw eggs decreased as storage time increased during storage at 4°C and 25°C. Eggshell strength is an important indicator of external quality of eggs. The external quality of eggs is mainly influenced by the absorption of calcium and phosphorus from the hens' diet (Küçükyilmaz et al., 2014). A previous study showed that even slight changes in dietary composition can significantly affect eggshell breakage (Hamilton et al., 1979). In the present study, neither dietary ASTA level nor storage time affected eggshell strength.

The rate of weight loss is an important indicator for evaluating the freshness of eggs, which is directly related to the economic value of eggs (Hidalgo et al., 1996; Wardy et al., 2013a,b). In previous studies, storage time and temperature were shown to remarkably affect weight loss of eggs (Silversides and Scott, 2001; Hammershoj et al., 2008). Furthermore, with increasing storage time at 28°C, weight loss of eggs increased and yolk index and HU decreased (Samli et al., 2005). In the present study, weight loss of eggs increased with storage time, but dietary ASTA level did not affect weight loss of eggs.

Our study indicated that with increasing storage time at 4°C and 25°C, yolk index and HU decreased, whereas yolk pH, yolk color, and weight loss of eggs increased, which was similar to previous results (Caner and Cansiz, 2008; Wardy et al., 2013a,b; Wang et al., 2015a,b). Moreover, a previous study showed that feeding 1.35% ASTA (from algae) did not affect production performance or egg quality except for egg yolk color (Walker et al., 2012). Our results demonstrated that dietary ASTA level had no effect on yolk index, yolk pH, or HU. Interestingly, we found interactions between dietary ASTA level and storage time on yolk index, yolk color, and HU, which improved the internal quality of eggs during storage. Previously, ASTA was used successfully to increase pigmentation of egg yolks or poultry meat (Takahashi et al., 2004; Walker et al., 2012). Feeding ASTA deepened the yolk color of raw eggs, which may be related to efficient sedimentation of ASTA.

Yolk index is an indicator of the spherical shape of the egg yolk, which indicates the freshness of an egg (Damir et al., 2014). During storage of eggs, the yolk index decreases because of a gradual weakening of the vitelline membrane as the egg yolk absorbs water from the albumen (Obanu and Mpieri, 1984; Alyssa et al., 1996; Wang et al., 2015a,b). The present study showed that the decline in yolk index was delayed in the high-dose groups (80 and 160 mg/kg ASTA) until 6 or 8 wk of storage at 4°C. Astaxanthin can scavenge free radicals by

Table 7. Effect of storage time on quality of eggs stored at 25°C.[1]

	Storage time (wk)						*P*-value		
Item	0	2	4	6	8	SEM	ANOVA	Linear	Quadratic
Yolk index	0.51^{a}	0.37^{b}	0.25^{c}	0.18^{d}	0.15^{e}	0.011	<0.001	<0.001	<0.001
Yolk color	12.92	12.97	13.46	-*	-*	0.205	0.511	0.290	0.609
Yolk pH	6.00^{d}	6.33^{c}	6.52^{b}	6.72^{a}	6.76^{a}	0.030	<0.001	<0.001	<0.001
Weight loss (%)	0.00^{e}	0.63^{d}	6.63^{c}	11.45^{b}	15.35^{a}	0.508	<0.001	<0.001	<0.001
Haugh unit	83.06^{a}	55.40^{b}	36.65^{c}	-*	-*	2.145	<0.001	<0.001	0.001
Eggshell strength (N/cm^{2})	41.94	41.87	41.89	42.23	42.36	0.288	0.976	0.554	0.771

[a–e]Mean values within a row without common superscripts differ significantly ($P < 0.05$).
*Not determined because the Haugh unit was very low (<25) and yolk breakage.
[1]Data were analyzed by 1-way ANOVA with orthogonal linear and quadratic contrasts.

Table 8. Effect of dietary natural astaxanthin levels on the concentrations of astaxanthin in yolk[1].

	Natural astaxanthin (mg/kg)						*P*-value		
Item	0	20	40	80	160	SEM	ANOVA	Linear	Quadratic
Astaxanthin concentration (mg/kg)	0.47[e]	5.47[d]	15.67[c]	34.30[b]	48.31[a]	3.400	<0.001	<0.001	<0.001

[a–e]Mean values within a row without common superscripts differ significantly ($P < 0.05$).
[1]Data were analyzed by 1-way ANOVA with orthogonal linear and quadratic contrasts.

auto-oxidation (Liang et al., 2009), which is related to degradation of carotenoids to transfer the excited state electrons of singlet oxygen to the carotenoid chain (Fleischmann et al., 2020; Kumar et al., 2020). Increasing the activity of antioxidant enzymes (e.g., glutathione peroxidase) in the yolk and albumen can improve the antioxidant status of eggs (Pappas et al., 2005). The unique structure of the ASTA terminal ring moiety can improve antioxidant activity and reduce cell membrane fluidity by binding to phospholipids of the cell membrane (Goto et al., 2001). In the present study, the delay in yolk index decline may have been related in part to the increased tenacity and reduced deformation of the vitelline membrane, caused in turn by the ASTA terminal ring moiety binding to the cell membrane (Goto et al., 2001). Furthermore, high-dose ASTA can effectively enhance the egg's antioxidant status through self-oxidation (Liang et al., 2009; Callie et al., 2018). However, in the present study, 160 mg/kg ASTA did not improve the yolk index in eggs stored at 25°C for 8 wk. The reason for the difference in yolk index results at 4°C and 25°C might be that internal oxidation of eggs occurs more rapidly at higher temperatures and ASTA cannot effectively prevent oxidative damage at higher temperatures (Koncsek et al., 2016).

The pH of a fresh egg yolk is 6 (Pike and Peng, 1988). During storage of eggs, yolk pH increases because of absorption of water from the albumen or lipid peroxidation of polyunsaturated fatty acids (Pike and Peng, 1988; Wang et al., 2015a,b). In the present study, the yolk pH of eggs increased with increasing storage time, consistent with results from a previous study (Mahmoud et al., 2010).

The HU score is calculated from the weight of an egg and the height of the thick albumen; it is in direct proportion to the viscosity of the thick white albumen (Chen et al., 1995). Storage conditions can affect HU values (Akyurek and Okur, 2009; Şekeroğlu et al., 2014). Our results showed that the HU values of eggs decreased during storage of eggs at 4°C and 25°C. In a previous study, supplementation with garlic (*Allium sativum*) was shown to improve the HU of eggs because of the increased antioxidant capacity (Mahmoud et al., 2010). However, we found that dietary ASTA levels did not affect HU of eggs during storage at 4°C and 25°C, which may be because dietary ASTA is mainly deposited in the yolk (Vargas et al., 2017). Interestingly, we found an interaction between dietary ASTA level and storage time on HU: we observed a remarkable increased in HU in the 160 mg/kg ASTA group compared with the control group in eggs stored at 4°C for 6 and 8 wk. These results may be related to the antioxidant capacity of ASTA. However, the mechanism underlying this interaction between dietary ASTA level and storage time on HU requires further study.

In summary, we demonstrated that dietary ASTA from *H. pluvialis* did not affect fresh egg quality. We found significant interactions between dietary ASTA level and storage time in terms of yolk index, yolk color, and HU at 4°C and 25°C. The results indicated that dietary ASTA delayed the decline in yolk index, yolk color, and HU during storage at 4°C and 25°C. On the basis of these results, we recommend dietary supplementation of 160 mg/kg ASTA. This information may provide an additional option by which to extend the storage time of eggs.

ACKNOWLEDGMENTS

This study was supported by Modern Agricultural Industry Technology System-Peking Poultry Innovation Team (BAIC04-2020), National Key R&D Program of China (2016YFD0700201), and 2020 Agricultural Science and Technology Rising Star Project (20200210). We thank Louise Adam, ELS(D), from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac) for editing the English text of a draft of this manuscript.

Conflict of Interest Statement: The authors have no conflicts of interest to declare. We confirm that this manuscript has not been published elsewhere and is not under consideration in whole or in part by another journal. All authors have approved the manuscript and agree with submission to poultry science.

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