



## Review Article

## Sources, dynamics in vivo, and application of astaxanthin and lutein in laying hens: A review

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## ABSTRACT

Astaxanthin (Ax) and lutein are important fat-soluble pigments and essential nutrients for human and animal health. *Haematococcus pluvialis* microalga and *Phaffia rhodozyma* yeast are ideal species for commercial Ax production. Marigold flowers are a main source of commercial lutein. Dynamics of dietary Ax and lutein in the gastrointestinal tract are similar to lipids, but their activities are tremendously challenged by many physiological and dietary factors; few data are available about these in poultry. Dietary Ax and lutein have insignificant effects on egg production and egg physical properties, but have pronounced effects on yolk color, nutrition, and functionality. The two pigments can also enhance antioxidative capacity and immune function of laying hens. A few studies have shown that Ax and lutein can improve fertilization and hatchability of laying hens. Considering the pigmentation and health benefits of Ax and lutein from hen feed to human food, the commercial availability, chicken yolk improvement, and immune function of Ax and lutein are the focuses of this review. The potential roles of carotenoids in the cytokine storm and gut microbiota are also briefly presented. The bioavailability, metabolism, and deposition of Ax and lutein in laying hens are suggested for future research.

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## 1. Introduction

Carotenoids are red, orange, and yellow pigments widely distributed in fruits, vegetables, fungi, flowers, and some kinds of animals (Liu et al., 2021a). Photosynthetic organisms can produce carotenoids through biosynthesis, whereas carotenoids found in the cells of humans and animals are only from the diet (Langi et al., 2018). Carotenoids are fat-soluble solids with more than 750 different structures (Rodriguez-Amaya, 2019). Based on their structures, carotenes and xanthophylls are two main subclasses of

carotenoids (Rodriguez-Amaya, 2016). Different carotenoid structures naturally possess different physical, chemical and functional properties. Astaxanthin (Ax), lutein, and zeaxanthin are the main subclasses of xanthophylls or oxycarotenoids, and the former two pigments are easily obtainable because they are more abundant in plants than zeaxanthin.

Astaxanthin, also known 3,3'-dihydroxy- $\beta,\beta'$ -carotene-4,4'-dione, naturally occurs in algae and microbes (Mularczyk et al., 2020). In contrast to Ax, lutein ( $\beta,\epsilon$ -carotene-3,3'-diol) is presented mainly in plants (Eisenhauer et al., 2017). Lutein acts as a color pigment in the human eye, along with zeaxanthin (Abdel-Aal et al., 2013). Besides the coloration, Ax and lutein have recently been demonstrated effective on antioxidation, immunity, and preventing some chronic diseases for humans and animals (Zafar et al., 2021). Natural Ax and lutein exist mainly in mono- or diesters whereas synthesized ones present as their free states. Natural Ax and lutein are considered more safe and reliable for use as nutraceuticals or colorants (Li et al., 2020; Stachowiak and Szulc, 2021).

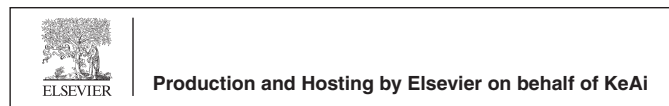
As known, Ax and lutein can be spread through the food chain. Animal-derived foods, including chicken eggs, salmon, and trout,

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although not usually the most abundant sources of Ax and lutein for humans, contain highly bioavailable and stable pigments (Bunger et al., 2014; Khalighi et al., 2021). Astaxanthin or lutein-enriched eggs have been found to increase their serum contents and mitigate hypertension (Bunger et al., 2014; Hatabu et al., 2020). Besides the yolk color, adding lutein or Ax to the diet of laying hens can generate significantly beneficial effects on antioxidative status, anti-inflammation, and immunity, all of which are desirable for both consumers and producers (Shevchenko et al., 2020). Astaxanthin supplementation in the laying hen's diet appreciably affected the egg yolk color and conferred a better accumulation of total Ax and cis-isomers into eggs as the supplementation dose increased (Dansou et al., 2021).

The properties and application of Ax and lutein for human health and nutrition have been updated regularly; however, for animals, related information is scarce. Only Raza et al. (2021) briefly reviewed the beneficial effects of Ax on broiler chickens and ruminants. Considering the global consumption of chicken eggs in the food chain, the natural enrichment in the yolk and most importantly the physiological functions of the two pigments, this paper reviewed the natural sources, dynamics in vivo, and application of Ax and lutein as feed additives in laying hens, with special interest in egg quality.

## 2. Sources of astaxanthin and lutein

### 2.1. Source of astaxanthin

Natural Ax can come directly from algae, bacteria, and yeast, and indirectly from fish and shellfish when they eat phytoplankton, also known as microscopic marine algae, the base of several aquatic food webs. Commercial Ax is mainly from algae and yeasts. *Haematococcus pluvialis* (*H. pluvialis*) exhibits a red color at its mature stage due to accumulated Ax. *H. pluvialis* has an excellent Ax content (55 mg/g of dry weight) and productivity, in particular, under environmental stress conditions (Grujić et al., 2022; Todorović et al., 2021). Other strains with high Ax yields include *Neochloris wimmeri*, *Protosiphon botryoides*, and *Scotiellopsis oocystiformis*, which can produce 15 mg/g of dry biomass of Ax on average (Barreiro and Barredo, 2018).

The Ax production capacity of *Phaffia rhodozyma* (*P. rhodozyma*) strains, red yeasts, has been reviewed by Mussagy et al. (2021),

which is up to 6.4 mg/g of dry weight. In contrast, bacteria, such as *Escherichia coli*, has a maximum Ax quantity of 6.2 mg/g of dry weight (Gong et al., 2020). Fish and shellfish contain an extensive range of the pigment from 4 mg/kg in salmon to 2.1 g/kg in *Portunus segnis* (Ambati et al., 2014; Hamdi et al., 2020). For comparison, yeasts and bacteria produce less Ax than algae. By literature analysis, algae species have the best production capacity of Ax by dry weight; therefore, microalgal Ax is a promising supplement for livestock and poultry production (Saadaoui et al., 2021; Trichet and Amaya, 2022). The summary of Ax sources and contents ( $\geq 20$  mg/g of dry weight) in algae is listed in Table 1.

### 2.2. Source of lutein

Lutein has a wide range of food sources in nature. It is a main component of plant pigments in yellow corn, vegetables, fruits, and flowers, especially rich in orange fruits and vegetables, including papaya, pumpkin, citrus, wolfberry, kale, spinach, and leek (Eisenhauer et al., 2017). Commercially, marigold is a significant source of natural lutein, with a proximate content at 0.24 mg/g in yellow-green flowers and 21 mg/g in orange ones (on a dry weight basis); the darker the color, the higher the content (Manzoor et al., 2022). In terms of existing forms, there is little free lutein in orange blossoms, mainly in the form of diesters (Barreiro and Barredo, 2018). The contents of lutein in algae are varied from 3 to 21 mg/g on a dry weight basis (Lin et al., 2015; Mary Leema et al., 2022; Ren et al., 2021). Generally, among algae species, *Chlorella* has a higher lutein content than *Auxenochlorella*, *Chlamydomonas*, *Coccomyxaonubensis*, and *Scenedesmus*. Algae have a faster growth rate and more free lutein than marigold flowers, and this may explain why marigold is presently a primary source of commercial lutein. The main sources and contents ( $\geq 10$  mg/g of dry weight) of lutein in algae and marigold are shown in Table 1.

## 3. Dynamics in vivo of astaxanthin and lutein

### 3.1. The dynamics of astaxanthin in the body

Astaxanthin dynamics in vivo, including absorption, transport, metabolism, and deposition, are shown in Fig. 1. Free Ax is susceptible to chemical changes under the conditions of the gastrointestinal tract (Chen et al., 2022). It can be metabolized or

**Table 1**  
Sources and contents of astaxanthin and lutein in algae and marigold.

Main source	Strain number	Yield, mg/g	Reference
Astaxanthin content ( $\geq 20$ mg/g of dry weight) in algae			
<i>Haematococcus pluvialis</i>	CCALA 840	55	Todorović et al. (2021)
<i>H. pluvialis</i>	CCALA1081	46	Grujić et al. (2022)
<i>H. pluvialis</i>	LUGU (KM115647.1)	36	Yu et al. (2021)
<i>H. pluvialis</i>	NIES-144	36	Ranjbar et al. (2008)
<i>H. pluvialis</i>	CCAP34/7	27	Harker et al. (1996)
<i>H. pluvialis</i>	UTEX 2505	25	Panutai et al. (2021)
<i>Scenedesmus quadricauda</i>	PUMCC 4.1.40	24	Rajput et al. (2021)
<i>H. pluvialis</i>	CCAP34/7	23	Orosa et al. (2001)
<i>H. pluvialis</i>	UTEX16	22	Zhang et al. (2009)
Lutein content ( $\geq 10$ mg/g of dry weight) in algae			
<i>Chlorella sorokiniana</i>	NIOT-2	21	Mary Leema et al. (2022)
<i>Scenedesmus</i> sp.	ANI-KL 8D	15	Low et al. (2022)
<i>C. sorokiniana</i>	Kh12	14	Patel et al. (2022)
<i>Chromochloris zofingiensis</i>	BKT1 (mutant)	14	Huang et al. (2018)
<i>C. sorokiniana</i>	FACHB-275	13	Zheng et al. (2022)
<i>Chlorella vulgaris</i>	UTEX 265	12	Gong and Bassi (2017)
<i>Parachlorella</i> sp.	JD-076	12	Heo et al. (2018)
<i>C. sorokiniana</i>	FZU60	11	Ma et al. (2020)
Lutein content ( $\geq 10$ mg/g of dry matter) in marigold ( <i>Tagetes erecta</i> L.)			
Marigold petals	PNG variety	21	Manzoor et al. (2022)
Marigold petals		10	Kashyap et al. (2022)

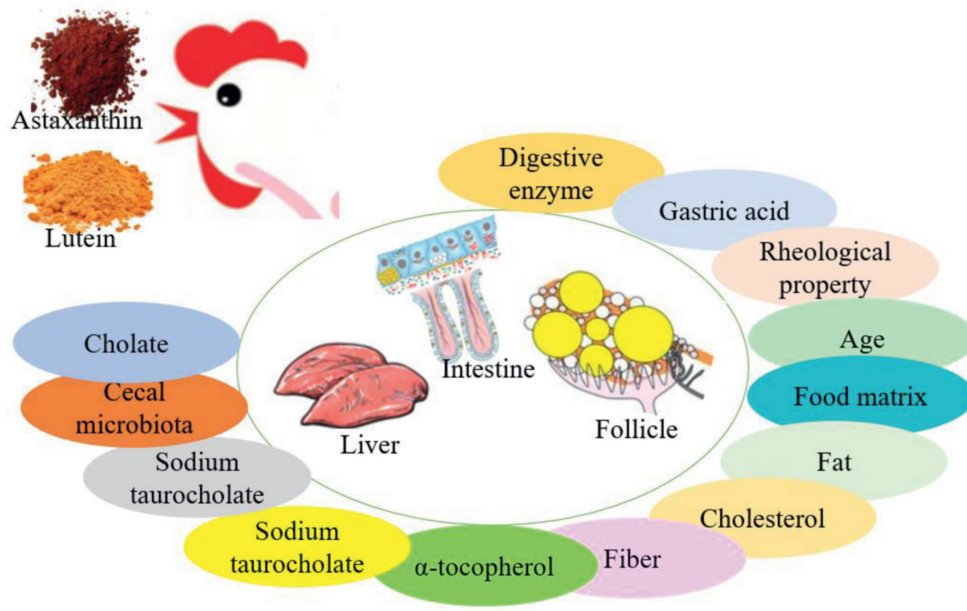


Fig. 1. Challenge factors involved in dynamics in vivo of astaxanthin and lutein.

decomposed into other molecular forms by digestive enzymes in epithelial cells. The strong acidic environment in the gastric cavity is also a huge challenge for maintaining Ax activity (Zhou and Wei, 2021). Additionally, the activity, delivery, and absorption of Ax can be compromised by the rheological properties of gastrointestinal tract. Therefore, how to safely transport free Ax into target sites via the digestive system has recently been a tough question (Chen et al., 2022). In contrast, natural Ax, which is dominated by its esters, is more stable and bioavailable under gastrointestinal conditions. A better understanding of Ax dynamics is essential to develop a better strategy to improve the in vivo retention and biological functions of Ax. However, the biochemistry and physiology involved in absorption, tissue deposition, and metabolism of Ax are not fully elucidated in poultry.

The absorption process for Ax is considered similar to that of lipids due to its fat-soluble properties, mainly involving disruption of the food matrix and molecular linkages, uptake in lipid droplets, micelle formation, and uptake from micelles into enterocytes and incorporation for transport into chylomicrons. Aside from the physiological factors of the gastrointestinal tract mentioned above, Ax absorption can be affected by dietary factors (food matrix, lipids, cholesterol, and fiber), cecal microbiota, and animal age (Li et al., 2022; Schmeisser et al., 2021). A decrease in gut microbiota can lead to a lower oral absorbability of Ax in the small intestine (Li et al., 2022). Fatty acid-mediated protein encapsulation can improve the water-solubility, storage stability, and oral absorption of Ax (Huang et al., 2022). Additionally, Ax absorption is negatively related to dietary fibers and positively related to cholesterol,  $\alpha$ -tocopherol, and sodium taurocholate (Zimmer et al., 2022). Likewise, Ax esters are easier to absorb than its free form. In the future, it will be interesting to compare the absorption efficiency among different sources as well as chemical forms of Ax.

After absorption from the gastrointestinal tract, the Ax is transported into the liver for metabolism and delivery to the other organs; of course, the remainder Ax is stored in the tissues. The storage or deposition potential of Ax varies in the liver, skin, fat, egg and muscle, with muscle appearing to have the lowest storage capacity (Schmeisser et al., 2021; Surai et al., 2016; Xi et al., 2022). Meanwhile, some Ax undergoes physiological decomposition. The

metabolism of carotenoids is involved in many oxidases and non-oxidases via random or nonrandom (double bonds) cleavages. Xanthophylls can only serve as a substrate for beta-carotene-9,10'-oxygenase, potentially giving rise to several 3-hydroxy metabolites depending on the side and number of cleavages (Giordano and Quadro, 2018). Astaxanthin carotenoid is metabolized into (rac)-3-hydroxy-4-oxo-beta-ionone and its reduced form (rac)-3-hydroxy-4-oxo-7,8-dihydro-beta-ionone independent of the xenobiotic-metabolizing enzymes in rat hepatocytes (Wolz et al., 1999). In rats, the first-pass extraction ratio of Ax in the liver (0.490) is lower than in the gastrointestinal tract (0.901), indicating that the liver is a primary metabolism site, and a related enzyme is cytochrome P-450 1A1/2 (Choi et al., 2011). It is deduced that poultry have a similar process for the absorption and metabolism of Ax, which needs further validation.

### 3.2. The dynamics of lutein in the body

As shown in Fig. 1, lutein has similar dynamics in vivo to Ax. In animals, the digestion and absorption of lutein is affected by physiological and dietary factors (Bhat and Mamatha, 2021; Tso et al., 2018). Before being absorbed by enterocytes, lutein esters are hydrolyzed by gastrointestinal enzymes, such as cholesterol esterase (Mrowicka et al., 2022). The absorbed lutein is mainly stored in the liver and deposits in fat, adrenal glands, skin, shell, and egg yolk. The main steps include release from food in the gastrointestinal tract, forming chylomicrons together with cholate, cholesterol, fatty acids, etc., transport from the intestinal mucosa through the lymphatic vessels to the blood and the liver, and finally transport from the liver through very low-density lipoproteins (VLDL) to the surrounding tissues (Schnebelen-Berthier et al., 2021; Tyssandier et al., 2002). VLDL, LDL, and high-density lipoprotein (HDL) are all involved in the transport of lutein (Britton et al., 2009). The blood concentration of lutein is highly dependent on its degree of esterification and intake level (Xiong et al., 2022).

Due to the underdeveloped intestinal lymphatic system in poultry, carotenoids are mainly transported through the portal vein system to the liver and then the ovary (Nimpf et al., 1989). Carotenoid deposition in the egg yolk is highly related to the VLDL

receptor, which can bind to VLDL and transport yolk precursors, including VLDL and vitellogenin, to the oocyte. The VLDL receptor plays a vital role in regulating egg production, yolk weight, and egg quality (Greco et al., 2022; Qian et al., 2012). Lutein is bonded and taken up by growing chicken oocytes via VLDL receptor-mediated endocytosis and ultimately deposits in the yolk (Cao and Wang, 2014). Like Ax, lutein is delivered to the liver and other tissues for deposition or further metabolism. Information about the metabolic processes of lutein is very limited in poultry, where 3'-oxolutein is the only known metabolite of lutein in chickens (Tyczkowski et al., 1986). Future work should focus on the bioavailability and metabolism of lutein as well as Ax in poultry.

#### 4. Application of astaxanthin in laying hens

##### 4.1. Astaxanthin and yolk indices

Supplementing Ax in the hen diet increased yolk color and Ax content. The effects of sources and dietary doses of Ax on the chicken egg yolk color and pigments contents are summarized in Table 2. Yolk color is an important sensory index for consumers (Sünder et al., 2022). The evaluation of the egg yolk pigmentation is performed by examining the appearance, the yolk color fan score, L\* (lightness), a\* (red), and b\* (yellow) values of the egg yolk. Studies showed that adding Ax dose-dependently improved the yolk color and had no negative effects on other indicators. Similarly, yolk color scores increased significantly with Ax supplementation from 0.48 to 23.9 at week 8 and 0.66 to 23.5 at week 24. Furthermore, Ax can fully affect a\* of the egg yolk and satisfy the consumer preference for the yolk color (Grashorn, 2016). Walker et al. (2012) found in an 8-week trial that color changed after Ax addition, peaked after 8 days of feeding, and stabilized over time. This may explain the similarity between the data at weeks 8 and 24, and the minimal change in yolk color with feeding duration (Dansou et al., 2021).

The increased yolk color is mainly from accumulated Ax, which accounts for 48.3 µg/g when hens are fed with a diet containing microalgal *H. pluvialis* at 160 mg/kg of feed after a 4-week feeding trial (Heng et al., 2021). Dietary Ax-rich corn (containing Ax at

24.3 mg/kg of diet) can lead to 14.2 µg/g of Ax in the yolk in a 3-week trial (Liu et al., 2021b). *Paracoccus carotinifaciens*, an aerobic Gram-negative bacterium, produces a carotenoid mixture that contains Ax as a main component. The dietary concentration of *P. carotinifaciens* at 8 mg/kg results in 6.65 µg/g of Ax in the yolk (Honda et al., 2021). Additionally, yeast *P. rhodozyma* and marine byproducts, especially cooked shrimp head, can also significantly increase the Ax content in the yolk. It can be seen from the above data that microalgae, bacteria, yeasts, Ax-rich corn, and marine byproducts can be used as suitable sources to produce biofortified Ax eggs. It is worth reminding that future research should focus on the relationships among Ax sources, dietary concentrations, deposition rates, and hen species.

The nutritional and functional components in the egg yolk mainly consist of lipids, proteins, and minerals. Besides coloring the egg yolk, dietary Ax can also affect the metabolism of lipids and fatty acids, which reflects its biological function. In the chicken egg yolk, the primary saturated fatty acids are palmitic acid and stearic acid. Shevchenko et al. (2020) found that Ax at a dose of 10 mg/kg of feed reduced the content of palmitoleic acid by increasing the proportion of cis-10-heptadecenoic acid, and at a dose of 30 mg/kg reduced the proportion of n-3 polyunsaturated fatty acids, including cis-4,7,10,13,16,19-docosahexaenoic acid, in the lipids of the yolk. Inclusion of red crab meal or krill meal in the rations of laying hens increased the n-3 and n-6 fatty acid content in eggs (Carrillo-Domínguez et al., 2005; Prommetta et al., 2020). Up to now, whether Ax influences proteins or minerals in the yolk is also unclear.

##### 4.2. Astaxanthin and other egg indices

The quality of the albumen and physical indices do not appear to be sensitive to dietary Ax. Most literature showed that albumen height, Haugh unit, eggshell thickness, and eggshell strength were unaffected by dietary Ax in laying hens (Conradie et al., 2018; Dansou et al., 2021; Heng et al., 2021; Honda et al., 2021). Only a few studies fortuitously found that dietary Ax increased eggshell weight (Zhu et al., 2021) and egg size (Conradie et al., 2018).

**Table 2**  
Summary of dietary astaxanthin and lutein effects on the chicken yolk color and pigment contents.

Item	Pigment	Hen			Yolk		Reference	
	Source	Dietary content, mg/kg	Species	Age, week	Consumption, week	Color		Pigment content, µg/g
Astaxanthin	<i>Haematococcus pluvialis</i>	40/80/160	Nongda No. 3		4	+	15.7/34.3/48.3	Heng et al. (2021)
	<i>H. pluvialis</i>	25/50/100	Hy-Line Brown	50	6	+	12.9/21.1/44.2	Gao et al. (2020)
	<i>H. pluvialis</i>	10/20/40/80	Shaver Leghorn	19	6	+	18.4/17.7/30.1/31.1	Magnuson et al. (2018)
	<i>H. pluvialis</i>	21.3/42.6	Hy-line Brown	20	12	+	9.85/23.5	Dansou et al. (2021)
	<i>H. pluvialis</i>	10/20/30	Hy-Line W36	23	12	+	19.9/18.4/15.4	Shevchenko et al. (2021)
	Astaxanthin-rich corn	24.3	Hy-Line Brown	28	4	+	14.2	Liu et al. (2021b)
	<i>H. pluvialis</i>	24.3	Hy-Line Brown	28	4	+	13.5	Liu et al. (2021b)
	<i>Paracoccus carotinifaciens</i>	8	Lohmann Julia Lite	91	3	+	6.65	Honda et al. (2021)
	Shrimp/scallop/squid head <sup>1</sup>		Bovans white	48	3	+	2.2/1.2/0.5	Toyes-Vargas et al. (2018)
	<i>Phaffia rhodozyma</i>	0.96/1.44/1.92	Lohmann Brown	60	6	+	undetected	Zhu et al. (2021)
Lutein	Marigold	2.86	Comb White Leghorns	4	4	+	49.4	Titcomb et al. (2019)
	Algal <i>D. tertiolecta</i>	2206	Hy-Line Brown	65	3	+	40	Kim and Shin (2022)
	Marigold	12/10 (non-/coated)	Hy-Line Brown	56	5	+	11.1/18.0	Wen et al. (2021)
	Yellow/orange corn	3.0/4.1	Novogen White	32	4	+	7.10/7.40	Ortiz et al. (2021)
	Stevia leaf	1.18/1.52	Hy-Line Brown	23	4	+	3.71/4.52	Pirgozliev et al. (2022)
	Red/orange carrot leaf	3.20/3.20	Comb White Leghorns	4	4	+	3.67/3.04	Titcomb et al. (2019)
	White corn	1.70	Comb White Leghorns	4	4		2.04	Titcomb et al. (2019)
	White corn	0.5	Novogen White	32	4		1.1	Ortiz et al. (2021)
	Shrimp/scallop/mackerel/squid <sup>1</sup>		Bovans white	48	3	+	7.2/8.8/4.6/7.8	Toyes-Vargas et al. (2018)

+, Color burn.

<sup>1</sup> Cooked marine byproduct meals, shrimp head, scallop viscera, whole mackerel, and squid viscera, added at 50 g/kg of feed.

Literature about the effects of Ax on the chemical composition of albumen and eggshell is unavailable.

#### 4.3. Astaxanthin and egg production

There are inconsistent effects of Ax and its source products on the production performance of laying hens. Zhu et al. (2021) found that dietary *P. rhodozyma* supplementation tended to increase feed intake and egg production, and the highest egg production was seen in the diet containing Ax at 1.2 g/kg; however, there were no significant differences in egg weight and feed conversion. However, Dansou et al. (2021) reported that Ax addition had no effect on egg weight and egg mass, but feed intake tended to increase. Compared to the white-corn based diet, *Paracoccus marcusii*-sourced Ax increased egg production, but did not influence the body weight of hens (Conradie et al., 2018). Marine byproducts, including scallop viscera, squid viscera, and shrimp head, added at 50 g/kg as dietary Ax sources affected egg laying rate, egg mass, and feed conversion ratio. Out of the marine sources, the dried scallop viscera had the highest egg laying rate, egg mass, and feed efficiency (Toyes-Vargas et al., 2018).

#### 4.4. Astaxanthin and antioxidation

The antioxidative properties of Ax are attributed to the presence of conjugated double bonds, the hydroxyl group, and the unsaturated ketone group at the end of the conjugated double bond chain, as well as the constitutive  $\alpha$ -hydroxy ketone from the hydroxyl and ketone groups. The molecular structural characteristics determine that Ax has an active electron to attract unpaired electrons from free radicals or actively provide electrons to free radicals, and thus effectively burst free radicals, especially single linear reactive oxygen species (Guan et al., 2019). Astaxanthin is a stronger antioxidant compared to  $\beta$ -carotene, vitamin E, and vitamin C, which are 54, 14 and 65 times stronger, respectively (Igielska-Kalwat et al., 2015). Indeed, research showed that Ax had a strong singlet oxygen quenching capacity, reduced the level of reactive oxygen species, and effectively suppressed lipid peroxidation (Hormozi et al., 2019). In the plasma, liver, and egg yolk, Ax increased the activities of superoxide dismutase, catalase, and glutathione peroxidase, but decreased malonaldehyde (an indicator of lipid peroxidation) in laying hens (Gao et al., 2020; Zhu et al., 2021).

The polyene chain in Ax traps radicals in the cell membrane, while the terminal ring of Ax can scavenge radicals at the outer and inner parts of the cell membrane (Ambati et al., 2014). With increasing dietary Ax, there is a linear increase in the ability to scavenge hydroxyl radical and superoxide anions, which may be related in part to the upregulated mRNA expression of genes encoding antioxidant enzymes and nuclear factor erythroid 2-related factor 2 in laying hens (Heng et al., 2021). Free Ax combined with oxygen radicals inhibited oxidation of free docosahexaenoic acid and improved the storage stability of docosahexaenoic acid-enriched eggs at 4 °C (Wang et al., 2022).

#### 4.5. Astaxanthin and immunity

The strong antioxidative capacity of Ax in theory and in practice predicts its immune function. Indeed, this has been well documented in human research. The related benefits of Ax to immunity include promoting immune mediator secretion, protecting immune cells and inhibiting inflammatory responses, and these are also proven in broilers (Cao and Wang, 2014). In laying hens, Zhu et al. (2021) reported that the content of serum IgG was significantly increased by Ax, but not for IgA and IgM, indicating that an appropriate amount of Ax is beneficial for innate immunity. Besides

being accumulated in the yolk, supplemental dietary microalgal Ax at 10, 20, 40, and 80 mg/kg seemed to be highly bioavailable for deposition in the plasma and liver of hens, and maximum levels in the plasma and liver reached 4.06  $\mu\text{g}/\text{mL}$  and 5.79  $\mu\text{g}/\text{g}$ , respectively (Magnuson et al., 2018). The high levels of circulating Ax will undoubtedly result in coordinated changes in intrinsic antioxidant systems and consequently the whole immune system. Furthermore, via absorption and transport, dietary Ax can break through the productive barrier into the yolk, and recent studies in rodents have shown that Ax can permeate through the blood-brain barrier to exert neuroprotective benefits (Chik et al., 2022; Fu et al., 2022). Therefore, Ax can also modulate immunity through the nervous system, as well as coordinating a humoral immune response.

Inflammation, as a transient phenomenon of immune processes, includes a mix of pro- and anti-inflammatory factors. It is thought that the major pro-inflammatory cytokines are interleukin (IL) 1 $\beta$ , IL6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), whereas IL1 receptor antagonist, IL4, IL6, IL10, IL11, and IL13 are typical anti-inflammatory cytokines (Samuel, 2020). The anti-inflammatory mechanisms of Ax target inflammatory factors and multiple signaling pathways. Astaxanthin has been shown to have a significant anti-inflammatory effect on neurological diseases, diabetes, gastrointestinal diseases, hepatic and renal diseases, and eye and skin disorders (Chang and Xiong, 2020). In hens, Tolba et al. (2020) reported that dietary Ax decreased serum pro-inflammatory factor TNF- $\alpha$ , but also lowered IL10, an anti-inflammatory cytokine. The information about the effect of Ax on inflammatory markers and associated signaling pathways is very limited, and requires further study.

More importantly, the cytokine storm can also be attenuated by Ax. A cytokine storm, also known as cytokine cascade and hypercytokinemia, is a potentially fatal immune reaction consisting of a positive feedback loop between cytokines and white blood cells, with highly elevated levels of various cytokines. In theory, the anti-inflammatory properties of Ax can predict its effect on the cytokine storm as reviewed by Darenskaya et al. (2021) and Talukdar et al. (2020). How Ax decreases the cytokine storm and consequently morbidity and mortality in farm animals will be an interesting topic for future research.

#### 4.6. Astaxanthin and metabolism

For metabolic parameters in laying hens, Shevchenko et al. (2021) reported that the use of Ax at 10 mg/kg in the diet increased serum glucose, phosphorus and calcium, and reduced serum cholesterol, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, but did not affect serum creatinine and protein. They also specified that an Ax dose of 20 mg/kg increased serum glucose and cholesterol, decreased calcium and alkaline phosphatase; whereas a dose of 30 mg/kg increased serum glucose, cholesterol and alkaline phosphatase, but reduced creatinine and aspartate aminotransferase. Furthermore, Ax from *H. pluvialis* added at 25, 50 or 100 mg/kg linearly increased HDL and VLDL, but decreased hematologic triglyceride, cholesterol and LDL, and linearly increased hepatic HDL, but not for other parameters in laying hens (Gao et al., 2020). Dietary Ax at 80 mg/kg decreased hepatic polyunsaturated fatty acids, but increased serum alanine aminotransferase (Magnuson et al., 2018). The fluctuation of tissue concentrations of cholesterol, calcium, alkaline phosphatase, creatinine, and aspartate aminotransferase in diets containing Ax needs to be studied further.

#### 4.7. Astaxanthin and fertility

The egg hatching rate is usually expressed as the percentage of total eggs hatched out. Decreasing the number of dead embryos is

critical for enhancing the hatching rate of fertilized eggs. It has been well documented that Ax can enhance antioxidation and immunity, such as quenching free radicals, protecting the body from oxidative damage, promoting the production of antibodies, and strengthening defense function, all of which provide a guarantee for egg fertility and hatchability. Bai (2021) reported that adding Ax at 15 g/kg to the feed of laying breeder hens improved the percentages of fertilization, hatchability, and healthy chicks. Maternal intake of Ax can improve hatchability of fertilized eggs stored at high temperature and there are interactions between Ax levels and the storage temperature (Saito and Kita, 2011). These results suggest that the lowered hatchability of eggs stored at high temperatures can be restored through maternal intake of dietary Ax, and the beneficial effects of Ax may be more effective at the early stage of embryonic development.

Astaxanthin can also increase egg fertilization by protecting the semen quality of breeder roosters (Najafi et al., 2020). Additionally, Ax ameliorated the aging in vitro of oocyte quality and improved the developmental competence of both fresh and vitrified porcine and bovine oocytes through its antioxidant properties (Jia et al., 2020; Xiang et al., 2021). Astaxanthin improved the developmental competence of in vitro-grown oocytes and modified the steroidogenesis of granulosa cells derived from bovine early antral follicles (Abdel-Ghani et al., 2019). Therefore, Ax may be beneficial for poultry artificial insemination, but data about this is poor.

#### 4.8. Astaxanthin and gut microbiota

It is well known that dietary nutrients, gut microbiota and the host interact in subtle ways. Astaxanthin has been shown to confer many benefits to the host through modulating gut health. Astaxanthin was found to affect gut microbiota composition, which is associated with reductions in local and systemic inflammation, oxidative stress, and improvement of metabolic homeostasis in mice (Wu et al., 2020). Furthermore, Ax prevented alcoholic fatty liver disease by modulating mouse gut microbiota (Liu et al., 2018). Astaxanthin from *H. pluvialis* alleviates obesity by modulating lipid metabolism and gut microbiota in mice fed a high-fat diet (Wang et al., 2021a). Astaxanthin alleviated ochratoxin A-induced cecum injury and inflammation in mice by regulating the diversity of cecal microbiota and toll-like receptor 4/myeloid differentiation factor 88/nuclear factor kappa-B signaling pathway (Chen et al., 2021b). Information about the effect of Ax on gut microbiota in food animals is scarce. Only Wang et al. (2021b) argued that intestinal microbiota biodiversity and richness were unaffected by dietary Ax from *P. rhodozyma* in shrimp. Therefore, how dietary Ax influences gut microbiota and the host, either directly or indirectly, in food animals deserves further study.

### 5. Application of lutein in laying hens

#### 5.1. Lutein and egg quality

The effect of sources and dietary doses of lutein on the chicken yolk color and pigment contents is summarized in Table 2. Dietary lutein is also a critical source for the yolk color. Studies showed that lutein increased the a\* and b\* value of the egg yolk and had no effect on the L\* value (Titcomb et al., 2019; Wen et al., 2021). A high level of lutein at 250 mg/kg reduced the L\* value (Englmaierová et al., 2013), but low levels at 10 to 40 mg/kg did not (Lokaewmanee et al., 2011). Likewise, Karadas et al. (2006) reported that adding marigold extract increased the scores of Roche color fan, and Skrivan et al. (2016) found that increasing doses of marigold flower extract increased the values of a\*, b\*, and the ratio of a\* and b\*, but decreased L\* value.

Dietary lutein from marigold can be accumulated in the yolk up to a level of 49.4 µg/g (Titcomb et al., 2019). Algal lutein from *Dunaliella* species added at 2.2 g/kg of diet can enrich lutein at about 40 µg/g in the yolk, equivalent to a 60 g/kg deposition rate (Kim and Shin, 2022). The extremely low deposition rate may be the main reason why algal source lutein is used less in practice, but more studies are needed. Fortunately, Demarco et al. (2022) suggested that cell disruption is a better method to increase the extractability of compounds and consequently enhance the bioavailability of algal Ax because the algal cell wall blocks digestion in animals. Additionally, stevia leaf, red-carrot leaf, and marine byproducts supplemented in the hen diet can have a significant coloring effect on the yolk, with maximum lutein content in the yolk measured at 4.52, 3.67 and 8.8 µg/g, respectively (Pirgozliev et al., 2022; Titcomb et al., 2019; Toyas-Vargas et al., 2018). Dried stevia leaf rich in lutein (50.4 mg/kg) supplemented at 10 and 20 g/kg to laying hens significantly increased the yolk color, but egg albumen, eggshell, and chemical compositions were not affected (Pirgozliev et al., 2022). Lutein is present in all distillery dry grain soluble samples and in all cases exceeded the content in corn (Shin et al., 2018). Diets containing 200 g/kg of corn distillery dry grain soluble fed to hens for 12 weeks enhanced yolk lutein content from 39.1 to 103 µg/g, with no effect on albumen and eggshell (Shin et al., 2016). Biofortified orange corn increased egg yolk pigmentation and lutein density of laying hens (Ortiz et al., 2021).

Yolk lipids can also be influenced by dietary lutein, but other egg parameters, including albumen height, Haugh unit, albumen pH, yolk pH, eggshell thickness, eggshell weight, egg dry matter, protein and minerals seem unresponsive to the dietary inclusion of lutein, except for fat. Grčević et al. (2019) found that marigold extract at 2 g/kg of feed increased total n-3 and docosahexaenoic acids with a most favorable ratio of n-6 to n-3. Additionally, yellow corn and red sorghum increased the concentrations of saturated and mono-unsaturated fatty acids in egg yolk (Rosa et al., 2017). Vegetables rich in lutein improved the albumen quality of chicken eggs (Obianwuna et al., 2022). The diet with added lutein or its original materials had no significant effects on shell-breaking strength, shell thickness, shell ratio, albumen ratio, and Haugh unit (Lokaewmanee et al., 2011; Titcomb et al., 2019; Pirgozliev et al., 2022). Ortiz et al. (2021) reported that the diet containing lutein-rich orange corn increased the Haugh unit compared to white corn.

#### 5.2. Lutein and egg production

The addition of non-saponified lutein from marigold flower meal and saponified lutein from marigold flower extract in the diet of Boris Brown laying hens at 10, 20, 30 and 40 mg/kg from 26 to 28 weeks of age had no significant effect on the egg mass, feed intake and egg weight (Lokaewmanee et al., 2011). Similarly, non-microencapsulated at 600 mg/kg (12 mg/kg available lutein) and microencapsulated lutein at 90.1 mg/kg (10 mg/kg available lutein) supplemented to laying hens (Hy-Line Brown, 54 weeks old) for 35 days did not affect the productive performance (Wen et al., 2021). Also, lutein differences in the diets based on white, yellow and orange corn did not cause significant changes in egg laying rates, egg weight and hen body weight (Ortiz et al., 2021). Additionally, marine byproducts with varied contents of lutein fed to hens increased yolk color but compromised egg production and feed efficiency (Toyas-Vargas et al., 2018). Inferentially, based on the literature, plant-derived dietary lutein is not enough to cause a change in egg production, but more studies are needed.

#### 5.3. Lutein and antioxidation

As previously reported, lutein is among the strongest of antioxidants, containing 11 conjugated double bonds, with a low

polarity, and exists in acyclic, monocyclic or bicyclic forms. Lutein is highly active against reactive oxygen species and free radicals, and is involved in photoprotection. Macular lutein is mainly localized in retinal membranes around Henle's fibers to filter against harmful short-wave blue light (Chae et al., 2021). Lutein, as a membrane antioxidant, is excellent to counter singlet oxygen and free radicals. Meanwhile, lutein has also been found to exert antioxidative effect in the adrenal glands, corpus callosum, lungs, testes, skin and brain. Of course, brain lutein, as a result of its antioxidative role, is considered protective to cognitive function (Beydoun et al., 2022).

In hens, marigold-derived carotenoids added at 60 mg/kg to the diet improved the color and singlet oxygen quenching activity of egg yolk (Kojima et al., 2022). Supplementing marigold extract at 1 and 2 g/kg did not influence the obtained results of lipid oxidation in yolks (Grčević et al., 2019). Diets consisting of marigold flower, dried basil herb and flowers of calendula and dandelion decreased lipid oxidation in laying hens (Kljak et al., 2021). Jang et al. (2014) reported that lutein promoted the accumulation of vitamin A and glycogen in the liver and lipid absorption, improving hepatic anti-oxidation. Chen et al. (2021a) demonstrated that the addition of marigold extract at 200 or 300 mg/kg of feed significantly increased the activities of superoxide dismutase and glutathione peroxidase, while malonaldehyde content was significantly reduced in a Chinese species of chickens. Similar results were found by Yuan (2009) in a global commercial species of laying hens.

More interestingly, like other carotenoids, lutein also has a salutary effect on the body, making it resistant and strong against chronic disease, and this, coupled with its conjugated double bond system, solidly lays the ground for its immune, anti-inflammatory and therapeutic potential in laying hens.

#### 5.4. Lutein and immune system

The structure and high chemical reactivity of lutein make it capable of exerting bioprotective effects on the body. Indeed, it is evident that lutein is involved in many beneficial processes, such as immune function, intercellular signaling pathways, cell cycles and gut microbiota; also, it helps against neurological disorders, eye diseases, cardiac complications, microbial infections, skin irritation, bone decay, etc (Mittra et al., 2021). Manochkumar et al. (2021) suggested that lutein provides neuroprotection by inhibition of neuro-inflammation and excitotoxic pathways, microglial activation, modulation of autophagy, attenuation of oxidative damage and activation of defensive antioxidant enzymes. The eye is regarded as an immune privileged site, where immune responses are reparative or protective, since cytokines released by immune cells compromise visual acuity by inducing inflammation and fibrosis. In the eye, circulating lutein is helpful to energy metabolism, proteostasis, antioxidant, anti-inflammation, innate immunity and longevity (Yamaguchi et al., 2022). Captive and caged animals easily become lutein deficient, which probably impairs the immune system and visual system and causes an early cull as a result, which may be a novel strategy to deepen the production capacity of farm animals.

Lutein can also regulate the response index of immunity and inflammation in hens. Gao et al. (2015) found that a supplemental xanthophylls mixture (lutein to zeaxanthin ratio = 40:60) decreased pro-inflammatory cytokines, IL1 $\beta$ , IL6, interferon  $\gamma$  and TNF- $\alpha$ , and increased anti-inflammatory cytokine expression of IL4 and IL10 in breeding hens and chicks. Chen et al. (2021a) reported that adding marigold extract at 200 or 300 mg/kg increased IgA, IgM and IgG in Taihang laying hens. Notably, the addition of lutein to commercial laying hens increased the Newcastle antibody titers, an allergen-specific antibody (Yuan, 2009). Additionally, Chung et al. (2017) observed that pre-treatment with lutein dose-dependently lowered lipopolysaccharide-induced secretion and

mRNA expression of IL6, IL1 $\beta$  and TNF- $\alpha$ . Given the active involvement of lutein in the processes of antioxidation, immunity and inflammation, it is putative that lutein is also capable of inhibiting a cytokine storm. Farm animals are the most susceptible population to a variety of viruses, which may result in a cytokine storm, but related literature is scarce.

#### 5.5. Lutein and metabolic parameters

The significant effects of dietary lutein on antioxidative activity and immunity will inevitably affect the metabolism of hens. However, few data are available which examine the effects of lutein supplementation on hematologic and hepatic parameters in hens. Adding lutein at 40 mg/kg in the diet of laying hens increased cyclooxygenase-2, inducible nitric oxide synthase, caspase-3, B cell lymphoma/leukemia-2 and HDL cholesterol in the liver, but did not increase serum triglycerides, cholesterol and LDL (Gao et al., 2018). Adding marigold flower meal did not affect the content of crude fat and cholesterol in the yolk of Hy-Line gray hens (Xin et al., 2022). Further studies are required to elucidate the effect of lutein on metabolomics and proteomics in hens.

#### 5.6. Lutein and fertility

Circulating and accumulated lutein in the body also benefits the reproductive function of hens. Specifically, yolk lutein is not a color aesthetic but rather one of the protectors of egg hatchability. Lutein in the yolk is closely related to embryonic development, determining the survival rate of the embryo and the immune capacity of the offspring (Surai et al., 2001). Lutein alleviated arsenic-induced reproductive toxicity via nuclear factor erythroid 2-related factor 2 signaling in male mice (Li et al., 2016). Lutein administered at 125 mg/kg increased the number of oocytes, embryo quality, fertilization rate, and 2-cell blastocysts in a mice model with polycystic ovary syndrome (Bandariyan et al., 2021). Maternal carotenoids throughout pregnancy can benefit the infant's carotenoid status (Addo et al., 2021). Additionally, eggs with an increased lutein content have a high lysozyme activity, an active antibacterial enzyme, so lutein can enhance antibacterial capacity during egg incubation and then improve the hatching rate (Cucco et al., 2007). Interestingly, lutein can vertically spread from parent to offspring in both birds and mammals, via dietary lutein or egg injection to improve the fertilization rates and hatching rates of animal breeders, especially broiler breeders. Although few data about this is available, it is recommended that lutein administration to improve farm animal reproduction be investigated as a future application prospect.

#### 5.7. Lutein and gut microbiota

Recently, the research has also focused on interactions between carotenoids and gut microbiota. Increasing total carotenoids was associated with higher gut bacterial diversity and greater abundance of some genera relevant to microbial macronutrient metabolism (Frankenfeld et al., 2022). A dominant population of *Bifidobacteria* and *Lactobacilli* in the gut microbiota can contribute to a good health status. Dietary supplementation of good prebiotics rich in lutein significantly increased populations of *Bifidobacteria* and *Lactobacilli*, reduced opportunistic pathogens, *Bacteroides* spp and *Clostridium* spp, and also decreased the activity of beta-glucuronidase, an enzyme involved in colorectal carcinogenesis (Molan et al., 2014). Additionally, many observational studies have shown the potential role of carotenoid supplementation in lowering the risk of progression of dysbiosis, leaky gut and low-grade inflammation (Rinninella et al., 2018).

Furthermore, microbiota dysbiosis and leaky gut can induce low-grade or acute inflammation, which seriously compromises the health and production performance of farm animals, particularly captive and caged animals. Coupled with the prohibition of growth-promoting antibiotics in many countries, this is becoming a deadly threat to animal production. Even if poor data are available about the influence of lutein on gut health in farm animals, it is plausible to suggest that carotenoids with strong antioxidative properties are capable of protecting gut health via modulating the gut microbiota.

## 6. Conclusions and outlook

Astaxanthin and lutein are the most abundant pigments in plants and the main sources of yolk color. Dietary supplementation of Ax and lutein can improve antioxidation, anti-inflammation, immunity and the metabolism of laying hens. Bioavailability and metabolism in vivo of Ax and lutein are the major problems for their current application in laying hens. Future application prospects will involve egg biofortification, gut microbiota, fertility, hatchability and related mechanisms of Ax and lutein in laying hens. Additionally, the visual and neuro-protective properties of the two pigments are suggested for application in animal breeders to avoid an early cull.

## Author contributions

**Hanyi Shi:** Writing - review & editing. **Xuejuan Deng:** Conceptualization. **Xiaoyu Ji:** Drawing the figure and checking references. **Ning Liu:** Writing and revising. **Huiyi Cai:** Supervision.

## Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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## References

Abdel-Aal el-SM, Akhtar H, Zaheer K, Ali R. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. *Nutrients* 2013;5:1169–85.

Abdel-Ghani MA, Yanagawa Y, Balboula AZ, Sakaguchi K, Kanno C, Katagiri S, et al. Astaxanthin improves the developmental competence of *in vitro*-grown oocytes and modifies the steroidogenesis of granulosa cells derived from bovine early antral follicles. *Reprod Fertil Dev* 2019;31:272–81.

Addo EK, Gorusupudi A, Allman S, Bernstein PS. The Lutein and zeaxanthin in pregnancy (L-ZIP) study-carotenoid supplementation during pregnancy: ocular and systemic effects-study protocol for a randomized controlled trial. *Trials* 2021;22(1):300.

Ambati RR, Phang SM, Ravi S, Aswathanarayana RG. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. *Mar Drugs* 2014;12(1):128–52.

Bai J. Effect of astaxanthin supplementation on egg hatching. *J China Feed* 2021;2021:52–5 [in Chinese].

Bandariyan E, Mogheseh A, Ahmadi A. The effect of lutein and *Urtica dioica* extract on *in vitro* production of embryo and oxidative status in polycystic ovary syndrome in a model of mice. *BMC Complement Med Ther* 2021;21:55.

Barreiro C, Barredo JL. Carotenoids production: a healthy and profitable industry. *Methods Mol Biol* 2018;1852:45–55.

Beydoun MA, Beydoun HA, Fanelli-Kuczmariski MT, Weiss J, Hossain S, Canas JA, et al. Association of serum antioxidant vitamins and carotenoids with incident alzheimer disease and all-cause dementia among US adults. *Neurology* 2022;98:e2150–62.

Bhat I, Mamatha BS. Genetic factors involved in modulating lutein bioavailability. *Nutr Res* 2021;91:36–43.

Britton G, Liaaen-Jensen S, Pfander H. Carotenoids: volume 5: nutrition and health. Birkhäuser Basel 2009. <https://doi.org/10.1007/978-3-7643-7501-0>.

Bunger M, Quataert M, Kamps L, Versloot P, Hulshof PJ, Togtema A, et al. Bioavailability of lutein from a lutein-enriched egg-yolk beverage and its dried resuspended versions. *Int J Food Sci Nutr* 2014;65:903–9.

Cao J, Wang W. Effects of astaxanthin and esterified glucosmannan on hematological and serum parameters, and liver pathological changes in broilers fed aflatoxin-B<sub>1</sub>-contaminated feed. *Anim Sci* 2014;85(2):150–7.

Carrillo-Domínguez S, Carranco-Jauregui ME, Castillo-Domínguez RM, Castro-González MI, Avila-González E, Pérez-Gil F. Cholesterol and n-3 and n-6 fatty acid content in eggs from laying hens fed with red crab meal (*Pleuroncodes planipes*). *Poultry Sci* 2005;84:167–72.

Chae SY, Shin MC, Jeon S, Kang MS, Han DW, Hong SW. A Simple route to the complexation of lutein with reduced graphene oxide nanocarriers and antioxidant protection against blue light. *Int J Nanomed* 2021;16:6843–60.

Chang MX, Xiong F. Astaxanthin and its effects in inflammatory responses and inflammation-associated diseases: recent advances and future directions. *Molecules* 2020;25:5342.

Chen X, Sun E, Chen Y, Zang C, Wang D, Hao E, et al. Effect of marigold extract on production performance, egg quality, immune function and antioxidant capacity of Taihang chicken. *Chinese J Anim Nutr* 2021a;33:7160–8 [in Chinese].

Chen Y, Su W, Tie S, Zhang L, Tan M. Advances of astaxanthin-based delivery systems for precision nutrition. *Trends Food Sci Technol* 2022;127:63–73.

Chen Y, Zhao S, Jiao D, Yao B, Yang S, Li P, et al. Astaxanthin alleviates ochratoxin A-induced cecum injury and inflammation in mice by regulating the diversity of cecal microbiota and tlr4/myd88/nf-kb signaling pathway. *Oxid Med Cell Longev* 2021b;2021:8894491.

Chik MW, Mohd Affandi MMRM, Singh GKS. Detection of astaxanthin at different regions of the brain in rats treated with astaxanthin nanoemulsion. *J Pharm BioAllied Sci* 2022;14:25–30.

Choi HD, Kang HE, Yang SH, Lee MG, Shin WG. Pharmacokinetics and first-pass metabolism of astaxanthin in rats. *Br J Nutr* 2011;105:220–7.

Chung RWS, Leanderson P, Lundberg AK, Jonasson L. Lutein exerts anti-inflammatory effects in patients with coronary artery disease. *Atherosclerosis* 2017;262:87–93.

Conradie TA, Pieterse E, Jacobs K. Application of *Paracoccus marcusii* as a potential feed additive for laying hens. *Poultry Sci* 2018;97:986–94.

Cucco M, Guasco B, Malacarne G, Ottonelli R. Effect of β-carotene on adult immune condition and antibacterial activity in the egg of the Grey Partridge. *Perdix. Comp Biochem Physiol A Mol Integr Physiol* 2007;147:1038–46.

Dansou DM, Wang H, Nugroho RD, He W, Zhao Q, Tang C, et al. Effects of duration and supplementation dose with astaxanthin on egg fortification. *Poultry Sci* 2021;100:101304.

Darenskaya M, Kolesnikova L, Kolesnikov S. The association of respiratory viruses with oxidative stress and antioxidants. Implications for the COVID-19 pandemic. *Curr Pharmaceut Des* 2021;27:1618–27.

Demarco M, de Moraes JO, Matos AP, Derner RB, de Farias NF, Tribuzi G. Digestibility, bioaccessibility and bioactivity of compounds from algae. *Trends Food Sci Technol* 2022;121:114–28.

Eisenhauer B, Natoli S, Liew G, Flood VM. Lutein and zeaxanthin-food sources, bioavailability and dietary variety in age-related macular degeneration protection. *Nutrients* 2017;9:120.

Englmaierová M, Skřivan M, Bubancová I. A comparison of lutein, spray-dried Chlorella, and synthetic carotenoids effects on yolk colour, oxidative stability, and reproductive performance of laying hens. *Czech J Anim Sci* 2013;58:412–9.

Frankenfeld CL, Hullar MAJ, Maskarinec G, Monroe KR, Shepherd JA, Franke AA, et al. The gut microbiome is associated with circulating dietary biomarkers of fruit and vegetable intake in a multiethnic cohort. *J Acad Nutr Diet* 2022;122:78–98.

Fu M, Liang X, Zhang X, Yang M, Ye Q, Qi Y, et al. Astaxanthin delays brain aging in senescence-accelerated mouse prone 10: inducing autophagy as a potential mechanism. *Nutr Neurosci* 2022;6:1–11.

Gao S, Li R, Heng N, Chen Y, Wang L, Li Z, et al. Effects of dietary supplementation of natural astaxanthin from *Haematococcus pluvialis* on antioxidant capacity, lipid metabolism, and accumulation in the egg yolk of laying hens. *Poultry Sci* 2020;99:5874–82.

Gao Y, Jin L, Peng H, Xu L, Wang Q, Ji J, et al. Xanthophylls increased HDLC level and nuclear factor PPAR $\gamma$ , RXR $\gamma$  and RAR $\alpha$  expression in hens and chicks. *J Anim Physiol Anim Nutr* 2018;102:e279–87.

Gao Y, Xie Q, Jin L, Sun B, Ji J, Chen F, et al. Supplementation of xanthophylls decreased proinflammatory and increased anti-inflammatory cytokines in hens and chicks. *Br J Nutr* 2015;114:1533.

Giordano E, Quadro L. Lutein, zeaxanthin and mammalian development: metabolism, functions and implications for health. *Arch Biochem Biophys* 2018;647:33–40.

Gong M, Bassi A. Investigation of *Chlorella vulgaris* UTEX 265 cultivation under light and low temperature stressed conditions for lutein production in flasks and the coiled tree photo-bioreactor (CTPBR). *Appl Biochem Biotechnol* 2017;183:652–71.

Gong Z, Wang H, Tang J, Bi C, Li Q, Zhang X. Coordinated expression of astaxanthin biosynthesis genes for improved astaxanthin production in *Escherichia coli*. *J Agric Food Chem* 2020;68:14917–27.

- Grashorn M. 14-Feed additives for influencing chicken meat and egg yolk color. handbook on natural pigments in food and beverages 2016. <https://doi.org/10.1016/B978-0-08-100371-8.00014-2>.
- Grčević M, Kralik Z, Kralik G, Galović O. Effects of dietary marigold extract on lutein content, yolk color and fatty acid profile of omega-3 eggs. *J Sci Food Agric* 2019;99:2292–9.
- Greco LSL, Stumpf L, Timpanaro S, Abril RC, Matías L, Ariadna B, et al. Impact of low-cost diets on maturation of the red claw crayfish *Cherax quadricarinatus*: an integrative approach during a long-term study. *Aqua* 2022;561:738614.
- Grujić VJ, Todorović B, Kranvogel R, Ciringier T, Ambrožić-Dolinsek J. Diversity and content of carotenoids and other pigments in the transition from the green to the red stage of *Haematococcus pluvialis* microalgae identified by HPLC-DAD and LC-QTOF-MS. *Plants* 2022;11:1026.
- Guan L, Liu J, Yu H, Tian H, Wu G, Liu B, et al. Water-dispersible astaxanthin-rich nanopowder: preparation, oral safety and antioxidant activity *in vivo*. *Food Funct* 2019;10:1386–97.
- Hamdi M, Nasri R, Dridi N, Li S, Nasri M. Development of novel high-selective extraction approach of carotenoproteins from blue crab (*Portunus segnis*) shells, contribution to the qualitative analysis of bioactive compounds by HR-ESI-MS. *Food Chem* 2020;302:125334.
- Harker M, Tsavalos AJ, Young AJ. Autotrophic growth and carotenoid production of *Haematococcus pluvialis* in a 30-liter air-lift photobioreactor. *J Ferment Bioeng* 1996;82:113–8.
- Hatabu T, Harada T, Takao Y, Thi DH, Yamasato A, Horiuchi T, et al. Daily meal supplemented with astaxanthin-enriched yolk has mitigative effects against hypertension in spontaneously hypertensive rats. *Biol Pharm Bull* 2020;43:404–8.
- Heng N, Gao S, Chen Y, Wang L, Li Z, Guo Y, et al. Dietary supplementation with natural astaxanthin from *Haematococcus pluvialis* improves antioxidant enzyme activity, free radical scavenging ability, and gene expression of antioxidant enzymes in laying hens. *Poultry Sci* 2021;100:101045.
- Heo J, Shin DS, Cho K, Cho DH, Lee YJ, Kim HS. Indigenous microalga *Parachlorella* sp. JD-076 as a potential source for lutein production: optimization of lutein productivity via regulation of light intensity and carbon source. *Algal Res* 2018;33:1–7.
- Honda M, Kawashima Y, Hirasawa K, Uemura T, Sun J, Hayashi Y. Astaxanthin Z-isomer-rich diets enhance egg yolk pigmentation in laying hens compared to that in all-E-isomer-rich diets. *Anim Sci J* 2021;92:e13512.
- Hormozi M, Ghoreishi S, Baharvand P. Astaxanthin induces apoptosis and increases activity of antioxidant enzymes in LS-180 cells. *Artif Cell Nanomed Biotechnol* 2019;47:891–5.
- Huang L, Li D, Ma Y, Liu Y, Liu G, Wang Y, et al. Dietary fatty acid-mediated protein encapsulation simultaneously improving the water-solubility, storage stability, and oral absorption of astaxanthin. *Food Hydrocolloids* 2022;123:107152.
- Huang W, Lin Y, He M, Gong Y, Huang J. Induced high-yield production of zeaxanthin, lutein, and  $\beta$ -carotene by a mutant of *Chlorella zofingiensis*. *J Agric Food Chem* 2018;66:891–7.
- Igielska-Kalwat J, Gościńska J, Nowak I. Karotenoidy jako naturalne antyoksydanty [Carotenoids as natural antioxidants]. *Postepy Hig Med Dosw* 2015;69:418–28.
- Jang I, Ko Y, Kang S, Ko Y, Kim S. Effects of dietary lutein sources on lutein-enriched egg production and hepatic antioxidant system in laying hens. *J Poultry Sci* 2014;51:58–65.
- Jia BY, Xiang DC, Shao QY, Zhang B, Liu SN, Hong QH, et al. Inhibitory effects of astaxanthin on postovulatory porcine oocyte aging *in vitro*. *Sci Rep* 2020;10:20217.
- Karadas F, Grammenidis E, Surai PF, Acamovic T, Sparks NH. Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. *Br Poultry Sci* 2006;47:561–6.
- Kashyap PK, Singh S, Singh MK, Gupta A, Tandon S, Shanker K, et al. An efficient process for the extraction of lutein and chemical characterization of other organic volatiles from marigold (*Tagetes erecta* L.) flower. *Food Chem* 2022;396:133647.
- Khalighi SM, Saraf-Bank S, Clayton ZS, Soltani S. A positive effect of egg consumption on macular pigment and healthy vision: a systematic review and meta-analysis of clinical trials. *J Sci Food Agric* 2021;101:4003–9.
- Kim MJ, Shin WS. Stability of zeaxanthin/lutein in yolk oil obtained from microalgae-supplemented egg under various storage conditions. *Lebensm Wiss Technol* 2022;155:112899.
- Kljak K, Carović-Stanko K, Kos I, Janječić Z, Kiš G, Duvnjak M, et al. Plant carotenoids as pigment sources in laying hen diets: effect on yolk color, carotenoid content, oxidative stability and sensory properties of eggs. *Foods* 2021;10:721.
- Kojima S, Koizumi S, Kawami Y, Shigeta Y, Osawa A. Effect of dietary carotenoid on egg yolk color and singlet oxygen quenching activity of laying hens. *J Poultry Sci* 2022;59:137–42.
- Langi P, Kiokias S, Vazarkas T, Proestos C. Carotenoids: from plants to food and feed industries. *Methods Mol Biol* 2018;1852:57–71.
- Li C, Gao Y, Huan Y, Ren P, Zhi J, Wu A, et al. Colon and gut microbiota greatly affect the absorption and utilization of astaxanthin derived from *Haematococcus pluvialis*. *Food Res Int* 2022;156:111324.
- Li LH, Lee JC, Leung HH, Lam WC, Fu Z, Lo ACY. Lutein supplementation for eye diseases. *Nutrients* 2020;12:1721.
- Li SG, Xu SZ, Niu Q, Ding YS, Pang LJ, Ma RL, et al. Lutein alleviates arsenic-induced reproductive toxicity in male mice via Nrf 2 signaling. *Hum Exp Toxicol* 2016;35:491–500.
- Lin JH, Lee DJ, Chang JS. Lutein production from biomass: marigold flowers versus microalgae. *Bioresour Technol* 2015;184:421–8.
- Liu C, Hu B, Cheng Y, Guo Y, Yao W, Qian H. Carotenoids from fungi and microalgae: a review on their recent production, extraction, and developments. *Bioresour Technol* 2021a;337:125398.
- Liu H, Liu M, Fu X, Zhang Z, Zhu L, Zheng X, et al. Astaxanthin prevents alcoholic fatty liver disease by modulating mouse gut microbiota. *Nutrients* 2018;10:1298.
- Liu X, Ma X, Wang H, Li S, Yang W, Nugroho RD, et al. Metabolic engineering of astaxanthin-rich maize and its use in the production of biofortified eggs. *Plant Biotechnol J* 2021b;19:1812–23.
- Lokawmanee K, Yamauchi K, Komori T, Saito K. Enhancement of yolk color in raw and boiled egg yolk with lutein from marigold flower meal and marigold flower extract. *J Poultry Sci* 2011;48:25–32.
- Low KL, Idris A, Yusof NM. An optimized strategy for lutein production via microwave-assisted microalgae wet biomass extraction process. *Process Biochem* 2022;121:87–99.
- Ma R, Zhang Z, Ho SH, Ruan C, Li J, Xie Y, et al. Twostage bioprocess for hyperproduction of lutein from microalga *Chlorella sorokiniana* FZU60: effects of temperature, light intensity, and operation strategies. *Algal Res* 2020;52:102119.
- Magnuson AD, Sun T, Yin R, Liu G, Tolba S, Shinde S, et al. Supplemental microalgal astaxanthin produced coordinated changes in intrinsic antioxidant systems of layer hens exposed to heat stress. *Algal Res* 2018;33:84–90.
- Manochkumar J, Doss CGP, El-Seedi HR, Efferth T, Ramamoorthy S. The neuroprotective potential of carotenoids *in vitro* and *in vivo*. *Phytomedicine* 2021;91:153676.
- Manzoor S, Rashid R, Prasad Panda B, Sharma V, Azhar M. Green extraction of lutein from marigold flower petals, process optimization and its potential to improve the oxidative stability of sunflower oil. *Ultrason Sonochem* 2022;85:105994.
- Mary Leema JT, Persia Jothy T, Dharani G. Rapid green microwave assisted extraction of lutein from *Chlorella sorokiniana* (NIOT-2) – process optimization. *Food Chem* 2022;372:131151.
- Mitra S, Rauf A, Tareq AM, Jahan S, Emran TB, Shahriar TG, et al. Potential health benefits of carotenoid lutein: an updated review. *Food Chem Toxicol* 2021;154:112328.
- Molan AL, Liu Z, Plimmer G. Evaluation of the effect of blackcurrant products on gut microbiota and on markers of risk for colon cancer in humans. *Phytother Res* 2014;28:416–22.
- Mrowicka M, Mrowicki J, Kucharska E, Majsterek I. Lutein and zeaxanthin and their roles in age-related macular degeneration-neurodegenerative disease. *Nutrients* 2022;14:827.
- Mularczyk M, Michalak I, Marycz K. Astaxanthin and other nutrients from *Haematococcus pluvialis*-multifunctional applications. *Mar Drugs* 2020;18:459.
- Mussagy CU, Pereira JFB, Dufossé L, Raghavan V, Santos-Ebinuna VC, Pessoa Jr A. Advances and trends in biotechnological production of natural astaxanthin by *Phaffia rhodozyma* yeast. *Crit Rev Food Sci Nutr* 2021;26:1–15.
- Najafi D, Taheri RA, Najafi A, Shamsollahi M, Alvarez-Rodriguez M. Effect of astaxanthin nanoparticles in protecting the post-thawing quality of rooster sperm challenged by cadmium administration. *Poultry Sci* 2020;99(3):1678–86.
- Nimpf J, Radosavljevic MJ, Schneider WJ. Oocytes from the mutant restricted ovulator hen lack receptor for very lowdensity lipoprotein. *J Biol Chem* 1989;264:1393–8.
- Obianwuna UE, Oleforuh-Okoleh VU, Wang J, Zhang HJ, Qi GH, Qiu K, et al. Natural products of plants and animal origin improve albumen quality of chicken eggs. *Front Nutr* 2022;9:875270.
- Orosa M, Valero JF, Herrero C, Abalde J. Comparison of the accumulation of astaxanthin in *Haematococcus pluvialis* and other green microalgae under N-starvation and high light conditions. *Biotechnol Lett* 2001;23:1079–85.
- Ortiz D, Lawson T, Jarrett R, Ring A, Scoles KL, Hoverman L, et al. Biofortified orange corn increases xanthophyll density and yolk pigmentation in egg yolks from laying hens. *Poultry Sci* 2021;100:101117.
- Panutai W, Boonpok S, Pornpukdeewattana S. Combination of mechanical and chemical extraction of astaxanthin from *Haematococcus pluvialis* and its properties of microencapsulation. *Biocatal Agric Biotechnol* 2021;33:101979.
- Patel AK, Vadrale AP, Tseng YS, Chen CW, Dong CD, Singhanian RR. Bioprospecting of marine microalgae from Kaohsiung Seacoast for lutein and lipid production. *Bioresour Technol* 2022;351:126928.
- Pirgooliev VR, Whiting IM, Kljak K, Mansbridge SC, Atanasov AG, Rose SP, et al. *Stevia* (*Stevia rebaudiana*) improves carotenoid content in eggs when fed to laying hens. *Foods* 2022;11:1418.
- Prommetta K, Attamangkune S, Ruangpanit Y. Krill meal enhances antioxidant levels and n-3 fatty acid content of egg yolk from laying hens fed a low-pigment diet. *J Poultry Sci* 2020;57:192–9.
- Qian S, Lei Q, Cao D, Wang C. A Review on effects of chicken yolk precursor and oocyte vitellogenesis receptor on egg quality. *J Food Sci* 2012;33:260–3.
- Rajput A, Singh DP, Khattar JS, Swatch GK, Singh Y. Evaluation of growth and carotenoid production by a green microalga *Scenedesmus quadricauda* PUMCC 4.1.40. under optimized culture conditions. *J Basic Microbiol* 2021. <https://doi.org/10.1002/jbom.202100285>.
- Ranjbar R, Inoue R, Shiraishi H, Katsuda T, Katoh S. High efficiency production of astaxanthin by autotrophic cultivation of *Haematococcus pluvialis* in a bubble column photobioreactor. *J Biochem Eng* 2008;39:575–80.

- Raza SHA, Naqvi SRZ, Abdelnour SA, Schreurs N, Mohammedsahle ZM, Khan I, et al. Beneficial effects and health benefits of astaxanthin molecules on animal production: a review. *Res Vet Sci* 2021;138:69–78.
- Ren Y, Sun H, Deng J, Huang J, Chen F. Carotenoid production from microalgae: biosynthesis, salinity responses and novel biotechnologies. *Mar Drugs* 2021;19:713.
- Rinninella E, Mele MC, Merendino N, Cintoni M, Anselmi G, Caporossi A, et al. The role of diet, micronutrients and the gut microbiota in age-related macular degeneration: new perspectives from the gut-retina axis. *Nutrients* 2018;10:1677.
- Rodriguez-Amaya DB. Structures and analysis of carotenoid molecules. *Subcell Biochem* 2016;79:71–108.
- Rodriguez-Amaya DB. Update on natural food pigments - a mini-review on carotenoids, anthocyanins, and betalains. *Food Res Int* 2019;124:200–5.
- Rosa AP, Bonilla CE, Londero A, Giacomini CB, Orso C, Fernandes MO, et al. Effect of broiler breeders fed with corn or sorghum and canthaxanthin on lipid peroxidation, fatty acid profile of hatching eggs, and offspring performance. *Poultry Sci* 2017;96:647–58.
- Saadaoui I, Rasheed R, Aguilar A, Cherif M, Jabri Al H, Sayadi S, et al. Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. *J Anim Sci Biotechnol* 2021;12:76.
- Saito F, Kita K. Maternal intake of astaxanthin improved hatchability of fertilized eggs stored at high temperature. *J Poultry Sci* 2011;48:33–9.
- Samuel PJ. Cytokine analysis: a fresh perspective. *J Interferon Cytokine Res* 2020;40:511–4.
- Schmeisser J, Verlhac-Trichet V, Madaro A, Lall SP, Torrisen O, Olsen RE. Molecular mechanism involved in carotenoid metabolism in post-smolt atlantic salmon: astaxanthin metabolism during flesh pigmentation and its antioxidant properties. *Mar Biotechnol* 2021;23:653–70.
- Schnebelen-Berthier C, Acar N, Simon E, Thabuis C, Bourdillon A, Mathiaud A, et al. The ALGOVUE clinical trial: effects of the daily consumption of eggs enriched with lutein and docosahexaenoic acid on plasma composition and macular pigment optical density. *Nutrients* 2021;13:3347.
- Shevchenko LV, Davydovych VA, Ushkalov VO, Midyk SV, Mykhalska VM. The effect of astaxanthin and lycopene on the content of fatty acids in chicken egg yolks. *Regul Mech Biosyst* 2020;11:568–71.
- Shevchenko LV, Iakubchak OM, Davydovych VA, Honchar VV, Ciorga M, Hartung J, et al. Influence of lycopene and astaxanthin in feed on metabolic parameters of laying hens, yolk color of eggs and their content of carotenoids and vitamin A when stored under refrigerated conditions. *Pol J Vet Sci* 2021;24:525–35.
- Shin EC, Shurson GC, Gallaher DD. Antioxidant capacity and phytochemical content of 16 sources of corn distillers dried grains with solubles (DDGS). *Anim Nutr* 2018;4:435–41.
- Shin HS, Kim JW, Kim JH, Lee DG, Lee S, Kil DY. Effect of feeding duration of diets containing corn distillers dried grains with solubles on productive performance, egg quality, and lutein and zeaxanthin concentrations of egg yolk in laying hens. *Poultry Sci* 2016;95:2366–71.
- Skrivan M, Englmaierová M, Skřivanová E, Bubancová I. Increase in lutein and zeaxanthin content in the eggs of hens fed marigold flower extract. *Czech J Anim Sci* 2016;60:87–96.
- Stachowiak B, Szulc P. Astaxanthin for the food industry. *Molecules* 2021;26:2666.
- Sünder A, Wilkens M, Böhm V, Liebert F. Egg yolk colour in organic production as affected by feeding – consequences for farmers and consumers. *Food Chem* 2022;382:131854.
- Surai PF, Fisinin VI, Karadas F. Antioxidant systems in chick embryo development. Part 1. Vitamin E, carotenoids and selenium. *Anim Nutr* 2016;2:1–11.
- Surai PF, Speake BK, Sparke NHC. Carotenoids in avian nutrition and embryonic development. 1. Absorption, availability and levels in plasma and egg yolk. *J Poultry Sci* 2001;38:1–27.
- Talukdar J, Bhadra B, Dattaroy T, Nagle V, Dasgupta S. Potential of natural astaxanthin in alleviating the risk of cytokine storm in COVID-19. *Biomed Pharmacother* 2020;132:110886.
- Titcomb TJ, Kaeppler MS, Cook ME, Simon PW, Tanumihardjo SA. Carrot leaves improve color and xanthophyll content of egg yolk in laying hens but are not as effective as commercially available marigold fortificant. *Poultry Sci* 2019;98:5208–13.
- Todorović B, Grujić VJ, Krajnc AU, Kranvogel R, Ambrožič-Dolinšek J. Identification and content of astaxanthin and its esters from microalgae *Haematococcus pluvialis* by HPLC-DAD and LC-QTOF-MS after extraction with various solvents. *Plants* 2021;10:2413.
- Tolba SA, Magnuson AD, Sun T, Lei XG. Dietary supplemental microalgal astaxanthin modulates molecular profiles of stress, inflammation, and lipid metabolism in broiler chickens and laying hens under high ambient temperatures. *Poultry Sci* 2020;99:4853–60.
- Toyes-Vargas E, Ortega-Pérez R, Espinoza-Villavicencio JL, Arellano-Pérez M, Civera R, Palacios E. Effect of marine by-product meals on hen egg production parameters, yolk lipid composition and sensory quality. *J Anim Physiol Anim Nutr* 2018;102:462–73.
- Trichet VV, Amaya E. 11 - astaxanthin use as carotenoid source and its benefits in feeds. In: Davis DA, editor. *Feed and feeding practices in aquaculture*. 2nd ed. Philadelphia: Woodhead Publishing Inc.; 2022. p. 309–35.
- Tso P, Vurma M, Ko CW, Lee D, DeMichele S. Effect of mono- and diglycerides on the digestion and absorption of lutein in lymph fistula rats. *Am J Physiol Gastrointest Liver Physiol* 2018;315:95–103.
- Tyczkowski JK, Schaeffer JL, Parkhurst C, Hamilton PB. 3'-Oxolutein, a metabolite of lutein in chickens. *Poultry Sci* 1986;65:2135–41.
- Tyssandier V, Choubert G, Grolier P, Borel P. Carotenoids, mostly the xanthophylls, exchange between plasma lipoproteins. *Int J Vitam Nutr Res* 2002;72:300–8.
- Walker LA, Wang T, Xin H, Dolde D. Supplementation of laying-hen feed with palm tocos and algae astaxanthin for egg yolk nutrient enrichment. *J Agric Food Chem* 2012;60:1989–99.
- Wang H, He W, Dansou DM, Zhang H, Nugroho RD, Tang C, et al. Astaxanthin improved the storage stability of docosahexaenoic acid-enriched eggs by inhibiting oxidation of non-esterified poly-unsaturated fatty acids. *Food Chem* 2022;381:132256.
- Wang M, Ma H, Guan S, Luo T, Zhao C, Cai G, et al. Astaxanthin from *Haematococcus pluvialis* alleviates obesity by modulating lipid metabolism and gut microbiota in mice fed a high-fat diet. *Food Funct* 2021a;12:9719–38.
- Wang W, Liu M, Fawzy S, Xue Y, Wu M, Huang X, et al. Effects of dietary *Phaffia rhodozyma* astaxanthin on growth performance, carotenoid analysis, biochemical and immune-physiological parameters, intestinal microbiota, and disease resistance in penaeus monodon. *Front Microbiol* 2021b;12:762689.
- Wen C, Su Y, Tao Z, Cheng Z, Zhou D, Wang T, et al. Dietary supplementation with microencapsulated lutein improves yolk color and lutein content in fresh and cooked eggs of laying hens. *J Poultry Sci* 2021;58:97–102.
- Wolz E, Liechti H, Notter B, Oesterheld G, Kistler A. Characterization of metabolites of astaxanthin in primary cultures of rat hepatocytes. *Drug Metab Dispos* 1999;27:456–62.
- Wu L, Lyu Y, Srinivasagan R, Wu J, Ojo B, Tang M, et al. Astaxanthin-shifted gut microbiota is associated with inflammation and metabolic homeostasis in mice. *J Nutr* 2020;150:2687–98.
- Xi L, Lu Q, Liu Y, Su J, Chen W, Gong Y, et al. Effects of fish meal replacement with *Chlorella* meal on growth performance, pigmentation, and liver health of largemouth bass (*Micropterus salmoides*). *Anim Nutr* 2022;10:26–40.
- Xiang DC, Jia BY, Fu XW, Guo JX, Hong QH, Quan GB, et al. Role of astaxanthin as an efficient antioxidant on the *in vitro* maturation and vitrification of porcine oocytes. *Theriogenology* 2021;167:13–23.
- Xin Q, Zhu Z, Miao Z, Hao X, Li L, Zhang L, et al. Effect of marigold flower dry meals on performance and egg quality of Hy-line gray hens. *Chinese J Anim Nutr* 2022;34:2896–906 [in Chinese].
- Xiong K, Zhao Y, Hu S, Ma A, Ma Y. Dose-response relationship between oral lutein intake and plasma lutein concentration: a randomized controlled trial. *Front Nutr* 2022;9:924997.
- Yamaguchi Y, Zampino M, Tanaka T, Bandinelli S, Moaddel R, Fantoni G, et al. The plasma proteome fingerprint associated with circulating carotenoids and retinol in older adults. *J Nutr* 2022;152:40–8.
- Yu C, Wang HP, Qiao T, Zhao Y, Yu X. A fed-batch feeding with succinic acid strategy for astaxanthin and lipid hyper-production in *Haematococcus pluvialis*. *Bioresour Technol* 2021;340:125648.
- Yuan N. Study on the effects of marigold extract and vitamin E on egg quality, antioxidant capacity and immune response. [Master Degree Thesis Dissertation]. Hebei Agricultural University; 2009 [in Chinese].
- Zafar J, Aqeel A, Shah FI, Ehsan N, Gohar UF, Moga MA, et al. Biochemical and immunological implications of lutein and zeaxanthin. *Int J Mol Sci* 2021;22:10910.
- Zhang BY, Geng YH, Li ZK, Hu HJ, Li YG. Production of astaxanthin from *Haematococcus* in open pond by two-stage growth one-step process. *Aqua* 2009;295:275–81.
- Zheng H, Wang Y, Li S, Wu Q, Feng X, Zheng Y, et al. Lutein production by microalgae using corn starch wastewater pretreated with rapid enzymatic hydrolysis. *Bioresour Technol* 2022;352:126940.
- Zhou Q, Wei Z. Food-grade systems for delivery of DHA and EPA: opportunities, fabrication, characterization and future perspectives. *Crit Rev Food Sci Nutr* 2021;2021:1–18.
- Zhu Y, Yin L, Ge J, Wu X, Peng Y, Zhang T, et al. Astaxanthin supplementation enriches productive performance, physiological and immunological responses in laying hens. *Anim Biosci* 2021;34:443–8.
- Zimmer TBR, Mendonça CRB, Zambiasi RC. Methods of protection and application of carotenoids in foods - a bibliographic review. *Food Biosci* 2022;48:101829.