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Effect of microencapsulated canthaxanthin and apo-ester on egg yolk color and antioxidant capacity in laying hens

Zhaoping Wang,* Xiaowei Sun,* Xinde Xu,† Di Zhou,† and Chao Wen*,[1]

*Department of Plant Protection, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, 210095, China; and †Department of Plant Protection, Zhejiang Medicine Co., Ltd Xinchang Pharmaceutical Factory, Shaoxing, 312500, China

ABSTRACT This study was conducted to evaluate the effects of common canthaxanthin (**CC**) or microencapsulated canthaxanthin (**MC**) combined with apo-ester (**AE**) on productive performance, egg yolk color and antioxidant capacity in laying hens. A total of 270 Hyline Brown laying hens at 56 wk of age were allocated to 3 groups with 6 replicates, and fed a wheat-soybean meal basal diet or the same diet supplemented with CC +AE or MC+AE at 5 mg/kg feed for each supplement. The productive performance was not affected by dietary treatments. The 2 test groups had higher ($P < 0.05$) yolk color score in fresh eggs than the control group, but the yolk color score of CC+AE group significantly declined ($P < 0.05$) with time, and a slight decline was also observed in the MC+AE group at 36 d. The MC +AE group had higher ($P < 0.05$) yolk color score of fried and boiled eggs than the other 2 groups. Higher ($P < 0.05$) feed canthaxanthin concentration was found in the MC+AE group at the end of experiment, which also had higher yolk canthaxanthin concentration in fresh eggs at 24 and 36 d as well as in fried, boiled and stored (4°C and 25°C) eggs. The 2 test groups had higher ($P < 0.05$) total antioxidant capacity in serum than the control group, and lower ($P < 0.05$) MDA content was observed in the MC+AE group. The mRNA level of cluster determinant 36 in jejunum was increased by the 2 test groups, and the same increase was also found in liver only in the MC+AE group. In conclusion, MC was more efficient in promoting yolk color and antioxidant capacity than CC when combined with AE.

Key words: canthaxanthin, apo-ester, laying hen, yolk color, antioxidant capacity

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INTRODUCTION

Egg yolk color is an important quality trait of eggs and it depends on the level of carotenoids in the yolk. Apart from their coloring effects, carotenoids are important for their antioxidant functions, i.e. scavenging free radicals and inducing antioxidant enzymes such as superoxide dismutase (**SOD**) and glutathione peroxidase (**GPX**) (Esatbeyoglu and Rimbach, 2017; Nabi et al., 2020; Pérez-Gálvez et al., 2020). Laying hens do not have the ability to synthesize carotenoids, diet is their only source of carotenoids. The commercial diet for laying hens does not contain enough carotenoids to obtain desirable pigmentation, so natural xanthophylls such as lutein and zeaxanthin, and synthetic carotenoids such as canthaxanthin and apo-ester (**AE**) are usually added to the diet to improve yolk color (Dansou et al., 2023). Canthaxanthin and AE are often added in combination to meet market needs (Englmaierová et al., 2013).

Carotenoids are insoluble in water and sensitive to oxidation because of their high degree of unsaturation. Various methods have been devised to improve their solubility and stability, in which microencapsulation technology is one of the most promising methods (Janiszewska-Turak, 2017). Microencapsulation serves as a tool to protect the sensitive compounds from oxidation by providing them with a coat material, which also has some other benefits, such as controlled release and taste masking (Tolve et al., 2021). Microencapsulation has been reported to improve the stability and bioavailability of lutein (Zhang et al., 2015; Zhao et al., 2018) and canthaxanthin (Arab et al., 2019; Hojjati et al., 2014). Our previous study showed that microencapsulation improved the pigmentation and deposition of lutein and canthaxanthin in egg yolk of laying hens (Wen et al., 2021; Wen et al., 2022).

Carotenoids are absorbed along with dietary lipids through passive diffusion or by transporters, such as cluster determinant 36 (**CD36**) and scavenger receptor class B type I (**SR-BI**) (Esatbeyoglu and Rimbach,

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[1]Corresponding author: wenchao@njau.edu.cn

2017). They are highly expressed in many tissues, particularly in liver and intestine (Shen et al., 2014). Dietary lipids and carotenoids are reported to regulate their expressions (Zhao et al., 2021; Liu et al., 2023). Therefore, the changes of their mRNA expression can be used to reflect the bioavailability of carotenoids (Desmarchelier and Borel. 2017). In addition, the mRNA expression of antioxidant enzymes can also be measured to elucidate the mechanism by which carotenoids regulate the synthesis of antioxidant enzymes.

This study was conducted to evaluate the effect of microencapsulated canthaxanthin (**MC**) plus AE on yolk color, canthaxanthin deposition and antioxidant capacity in laying hens. In addition, the mRNA expression of catorenoid transporters and antioxidant enzymes was determined in this study.

MATERIALS AND METHODS

Materials

The common canthaxanthin (**CC**) and MC were provided by Zhejiang Medicine Co., Ltd Xinchang Pharmaceutical Factory (Shaoxing, China). The CC was not microencapsulated, and MC was prepared using sodium lignosulfonate as the wall material, and the microencapsulation efficiency was above 95%. Briefly, canthaxanthin was finely dispersed in the matrix of sodium lignosulfonate to form an emulsion by high-pressure homogenization. Then it was spray-dried in a centrifugal atomizer at 120°C to form the microcapsule at a flow rate of 500 kg/h, which was further dried at 65°C in a fluidized bed. The AE microencapsulated with gelatin were provided by Guangzhou Leader Bio-technology Co., Ltd (Guangzhou, China).

Experimental Design, Diets and Husbandry

The procedures involving animals in this study were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee (SYXK [Su] 2017-0007).

A total of 270 Hyline Brown laying hens at 56 wk of age were used in this study. After 1 wk of adaptation, they were allocated to 3 groups with 6 replicates of 15 hens. The hens in the control group were fed a wheat-soybean meal basal diet (Table 1), and the rest hens were fed the basal diet supplemented with CC+AE (5 mg/kg each) or MC+AE (5 mg/kg each) for 36 d. An enzyme preparation composed of xylanase (45,000 U/g), β-glucanase (6,500 U/g), β-mannanase (2,500 U/g), pectinase (1,500 U/g), cellulase (1,500 U/g), α-amylase (6,500 U/g) and protease (8,000 U/g) was added to diet at 200 mg/kg. The enzyme preparation was provided by Jiangsu Yinong Bioengineering Co. Ltd (Suqian, China). The hens were allowed free access to mash feed and water, and they were exposed to a 16:8 light:dark cycle. Egg production and egg weight were recorded daily and feed consumption was recorded weekly per replicate. Feed conversion ratio were calculated.

Table 1. Ingredient composition and nutrient content of the basal diet (as-fed basis, g/kg unless otherwise stated).

Item	Content
Ingredient	
Wheat	698
Soybean meal	150
Soybean oil	20
Limestone	80
L-lysine	2
Premix[1]	50
Total	1,000
Calculated nutrient composition	
Metabolizable energy (MJ/kg)	11.05
Crude protein	161.4
Lysine	7.3
Methionine	3.7
Methionine + cystine	6.4
Calcium	42.4
Available phosphorus	3.1

[1]Premix supplied per kilogram of diet: transretinyl acetate, 11,000 IU; cholecalciferol, 3,500 IU; all-rac-α-tocopherol acetate, 20 mg; menadione, 1.5 mg; thiamin, 1 mg; riboflavin, 6 mg; nicotinamide, 40 mg; choline chloride, 350 mg; calcium pantothenate, 10 mg; pyridoxine. HCl, 2 mg; biotin, 0.04 mg; folic acid, 1 mg; cobalamin, 0.012 mg; Fe (ferrous sulfate), 60 mg; Cu (copper sulfate), 5 mg; Mn (manganese sulfate), 100 mg; Zn (zinc oxide), 65 mg; I (calcium iodate), 0.8 mg; Se (sodium selenite), 0.3 mg.

Sample Collection

On the first and last day of the experiment, 6 feed samples were taken from each group for the assay of dietary canthaxanthin concentration. During the experimental period, 2 fresh eggs per replicate was randomly collected for the evaluation of yolk color and canthaxanthin concentration every 12 d. At 36 d of the experiment, 8 eggs per replicate were randomly collected for the assessment of yolk color of fried, boiled and stored (4°C and 25°C) eggs (2 eggs per replicate for each assessment). Then 1 bird per replicate was randomly selected and killed by cervical dislocation. Blood (5 mL each) was taken from jugular vein and centrifuged at 3,000 × g for 15 min at 4°C. Then the serum was frozen at −20°C for further analysis. The jejunal mucosa and liver samples were taken and frozen in liquid nitrogen until analysis.

Yolk Color Assay

Yolk color of fresh, fried, boiled and stored eggs was evaluated by 2 individuals independently using a Roche yolk color fan, and average score was obtained. A 350 W electric egg cooker (JDQ-C3011, Guangdong Bears Electric Co. Ltd., Foshan, China) was used to fry eggs. Some soybean oil was poured into frying pan and preheated, and then eggs were broken into the frying pan, which was then covered with the lid and fried for 2.5 min on each side. Then yolk was separated and yolk color was evaluated. A 2200-W induction cooker (C22-WT2203, Midea Group Co. Ltd., Foshan, China) coupled with a stainless steel pot was used to boil eggs. Some water was poured into the cooker and heated until boiling, and then eggs were immersed in the boiling water for 10 min. After cooling down, eggs were cut

in half, and yolk color was evaluated. For the storing treatment, 2 eggs per replicate were stored at 4°C in a refrigerator or at 25°C in an incubator for 20 d. Then the yolk color of eggs was evaluated. Finally, the yolk mentioned above was collected and stored at −20°C until analysis.

Determination of Canthaxanthin Concentration

The canthaxanthin concentration in feed and egg yolk was measured by HPLC as previously described (Wen et al., 2022). Briefly, 1 g sample was dissolved in an extraction mixture composed of 10 mL hexane, 7 mL acetone, 6 mL ethanol and 7 mL methylbenzene. Then 2 mL of 40% KOH-methanol solution was added to saponify the samples in an ultrasonic water bath at 60°C for 20 min. After cooling down, 30 mL of hexane and 37 mL of 10% Na_2SO_4 solution were added and placed in darkness for 1 h. Finally, aliquots from upper phase were filtered through 0.45 μm membrane filter and used for HPLC injection. Canthaxanthin was chromatographically separated by C18 column (4.6 mm × 250 mm, 5 μm) using hexane-acetone (9:1, v/v) as the mobile phase at a flow rate of 1.2 mL/min, and the detection wavelength was set at 470 nm.

Determination of Serum Parameters

The concentrations of total protein (**TP**, A045-2), albumin (**ALB**, A028-2), glucose (**GLU**, A154-1), triglyceride (**TG**, A110-1) and total cholesterol (**TC**, A111-1) in serum were determined with analytical kits from Jiancheng Bioengineering Institute (Nanjing, China) using Olympus 2700 analyzer (Olympus, Tokyo, Japan). The assay of total antioxidant capacity (**T-AOC**, A015-3), total superoxide dismutase (**T-SOD**, A001-1), glutathione peroxidase (**GPX**, A005-1) and malondialdehyde (**MDA**, A003-1) in serum were performed using analytical kits from Jiancheng Bioengineering Institute.

Determination of mRNA Expression

Real-time PCR was used to determine the mRNA expression of carotenoid transporters, i.e. CD36 and SR-BI, and antioxidant enzymes, i.e. SOD1 and GPX1. Briefly, total RNA of samples was isolated using RNAiso reagent (TaKaRa Biotechnology, Dalian, China) and diluted in diethyl pyrocarbonate treated water to appropriate concentration. Then the diluted RNA was immediately reverse transcribed into cDNA with PrimeScript RT reagent Kit (TaKaRa), and the cDNA was quantified using SYBR Premix Ex Taq II (TaKaRa) on QuantStudio7 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA). The primers for target genes were designed according to the sequences located in GenBank, and β-actin was used to normalize the target genes (Table 2). Relative mRNA levels (arbitrary units) were calculated on the basis of PCR efficiency and threshold cycle (Ct) values as previously reported (Pfaffl, 2001). The mRNA level of each target gene for the control group was assigned a value of 1.

Statistical Analysis

All data were analyzed by 1-way ANOVA using SPSS 22.0 software (SPSS Inc., Chicago, IL). The differences among groups were examined by Duncan's multiple range test, which were considered to be significant at $P < 0.05$. The effects of time and egg treatments on yolk color and canthaxanthin concentration were also tested. Data were presented as means and standard error of means.

RESULTS

Productive Performance

The 2 test groups had numerically higher laying rate, egg weight, egg mass as well as numerically lower feed intake and feed conversion ratio than the control group, but no significant difference was observed (Table 3). Mortality was low and did not differ among groups (Data not shown).

Table 2. Sequences for real-time PCR primers.[1]

Gene	GeneBank ID	Primer sequence, sense/antisense	Product size (bp)
β-Actin	NM_205518	TGCTGTGTTCCCATCTATCG TTGGTGACAATACCGTGTTCA	150
CD36	NM_001030731.1	CTGGGAAGGTTACTGCGATT GCGAGGAACTGTGAAACGATA	178
SR-BI	XM_015275627.2	TCACTTCTACAATGCTGACCCAA TGAGCCATCAATGTATCCACTC	241
SOD1	NM_205064.2	CGGGCCAGTAAAGGTTACTGGAA TGTTGTCTCCAAATTCATGCACATG	83
GPX1	NM_001277853.3	ATGTTCGAGAAGTGCGAGGT ATGATGTACTGCGGGTTGGT	122

[1]Abbreviations: CD36, cluster determinant 36; SR-BI, scavenger receptor class B type I; SOD1, superoxide dismutase 1; GPX1, glutathione peroxidase 1.

Table 3. Productive performance of laying hens (n = 6 replicates).[1]

Item	Control	CC+AE	MC+AE	SEM	*P*-value
Laying rate, %	74.33	74.69	75.69	0.68	0.734
Egg weight, g	63.53	64.42	64.39	0.34	0.519
Egg mass, g	47.22	48.09	48.74	0.43	0.388
Feed intake, g/d	108.04	106.99	105.46	0.97	0.600
Feed conversion ratio	2.29	2.22	2.16	0.03	0.308

[1]Abbreviations: CC+AE, common canthaxanthin and apo-ester at 5 mg/kg each; MC+AE, microencapsulated canthaxanthin and apo-ester at 5 mg/kg each.

Yolk Color of Eggs

The wheat-soybean meal basal diet without pigments for the control group created a very light yellow yolk, the score of which was evaluated as 3 constantly (Table 4). For fresh eggs, the 2 test groups had higher ($P < 0.05$) yolk color score than the control group throughout the trial, and the MC+AE group had higher score than the CC+AE group at 24 and 36 d. The yolk color score of CC+AE group significantly declined ($P < 0.05$) with time, and a slight decline was also observed in the MC+AE group at 36 d. For fried, boiled and stored eggs, the MC + AE group had higher ($P < 0.05$) yolk color score than the other 2 groups. Fried and boiled eggs had lower ($P < 0.05$) yolk color score than the fresh eggs in each test group, whereas the stored eggs remained unchanged.

Feed and Yolk Canthaxanthin Concentration

Feed and yolk canthaxanthin concentration was not detected in the control group (Table 5). The feed canthaxanthin concentration at 1 d did not differ between the 2 test groups, but both of them significantly declined ($P < 0.05$) at 36 d, with the reduction being less in the MC+AE group. There was no difference in yolk canthaxanthin concentration of fresh eggs between the 2 test groups at 12 d, but only the CC+AE group showed a significant decrease ($P < 0.05$) at 24 and 36 d. The MC +AE group had higher ($P < 0.05$) yolk canthaxanthin concentration in fried, boiled and stored (4°C and 25°C) eggs than the CC+AE group. Compared with fresh eggs, the fried and boiled eggs had lower ($P < 0.05$) yolk canthaxanthin concentration in each group, but no difference was observed in stored eggs.

Table 4. Yolk color score in laying hens (n = 12 eggs).[1]

Item	Control	CC+AE	MC+AE	SEM	*P*-value
Fresh eggs					
12 d	3.00^{b}	13.94^{a,x}	14.00^{a,x}	0.71	<0.001
24 d	3.00^{c}	11.33^{b,y}	13.89^{a,x}	0.65	<0.001
36 d	3.00^{c}	7.11^{b,z}	13.61^{a,y}	0.62	<0.001
Fried eggs	3.00^{b}	3.17^{b,*}	5.17^{a,*}	0.29	<0.001
Boiled eggs	3.00^{c}	4.00^{b,*}	5.83^{a,*}	0.29	<0.001
Stored eggs (4°C)	3.00^{c}	8.75^{b}	14.00^{a}	1.36	<0.001
Stored eggs (25°C)	3.00^{c}	7.50^{b}	13.75^{a}	1.39	<0.001

$^{a\text{-}c}$Means within a row with different superscripts differ significantly at $P < 0.05$.

$^{x\text{-}z}$Means within a column with different superscripts differ significantly at $P < 0.05$.

*Means within a column with an asterisk differ significantly at $P < 0.05$ compared with fresh eggs at 36 d.

[1]Abbreviations: CC+AE, common canthaxanthin and apo-ester at 5 mg/kg each; MC+AE, microencapsulated canthaxanthin and apo-ester at 5 mg/kg each.

Table 5. Feed and yolk canthaxanthin concentration (mg/kg, n = 6 for feed and n = 12 for eggs).[1]

Item	Control	CC+AE	MC+AE	SEM	*P*-value
Feed					
1 d	ND	5.07^{x}	5.25^{x}	0.10	0.389
36 d	ND	1.04^{b,y}	4.20^{a,y}	0.48	<0.001
Fresh eggs					
12 d	ND	6.45^{x}	6.64	0.33	0.796
24 d	ND	3.28^{b,y}	6.24^{a}	0.59	0.004
36 d	ND	0.53^{b,z}	6.18^{a}	1.03	<0.001
Fried eggs	ND	0.24^{b,*}	3.34^{a,*}	0.50	<0.001
Boiled eggs	ND	0.33^{b,*}	4.14^{a,*}	0.67	<0.001
Stored eggs (4°C)	ND	0.56^{b}	6.14^{a}	0.96	<0.001
Stored eggs (25°C)	ND	0.52^{b}	6.26^{a}	0.93	<0.001

$^{a\text{-}c}$Means within a row with different superscripts differ significantly at $P < 0.05$.

$^{x\text{-}z}$Means within a column with different superscripts differ significantly at $P < 0.05$.

*Means within a row with an asterisk differ significantly at $P < 0.05$ compared with fresh eggs at 36 d.

[1]Abbreviations: ND, not detected; CC+AE, common canthaxanthin and apo-ester at 5 mg/kg each; MC+AE, microencapsulated canthaxanthin and apo-ester at 5 mg/kg each.

Serum Parameters

The contents of TP, ALB, GLU, TG and TC in serum did not differ among groups (Table 6). The 2 test groups had higher ($P < 0.05$) T-AOC than the control group, and lower ($P < 0.05$) MDA content was observed in the MC+AE group, whereas the activities of T-SOD and GPX showed no differences.

mRNA Expression

Compared with the control group, the 2 test groups had higher ($P < 0.05$) mRNA levels of CD36 in jejunum, and the same increase was also found in liver only in the MC+AE group (Figure 1). The mRNA levels of SR-BI,

Table 6. Serum parameters in laying hens (n = 6 replicates).[1]

Item	Control	CC+AE	MC+AE	SEM	*P*-value
TP (g/L)	48.69	50.15	48.92	1.98	0.954
ALB (g/L)	25.38	23.80	23.20	0.82	0.560
GLU (mmol/L)	12.05	12.78	11.46	0.33	0.281
TG (mmol/L)	6.94	9.09	7.08	0.57	0.241
TC (mmol/L)	3.16	3.08	2.83	0.12	0.561
T-AOC (U/mL)	4.19^{b}	4.87^{a}	4.99^{a}	0.15	0.044
T-SOD (U/mL)	382	396	404	8	0.552
GPX (U/mL)	1600	1668	1523	49	0.507
MDA (nmol/mL)	6.61^{a}	5.34^{ab}	4.22^{b}	0.36	0.014

$^{a\text{-}b}$Means within a row with different superscripts differ significantly at $P < 0.05$.

[1]Abbreviations: CC+AE, common canthaxanthin and apo-ester at 5 mg/kg each; MC+AE, microencapsulated canthaxanthin and apo-ester at 5 mg/kg each. TP, total protein; ALB, albumin; GLU, glucose; TG, triglyceride; TC, total cholesterol; T-AOC, total antioxidant capacity; T-SOD, total superoxide dismutase; GPX, glutathione peroxidase; MDA, malondialdehyde.

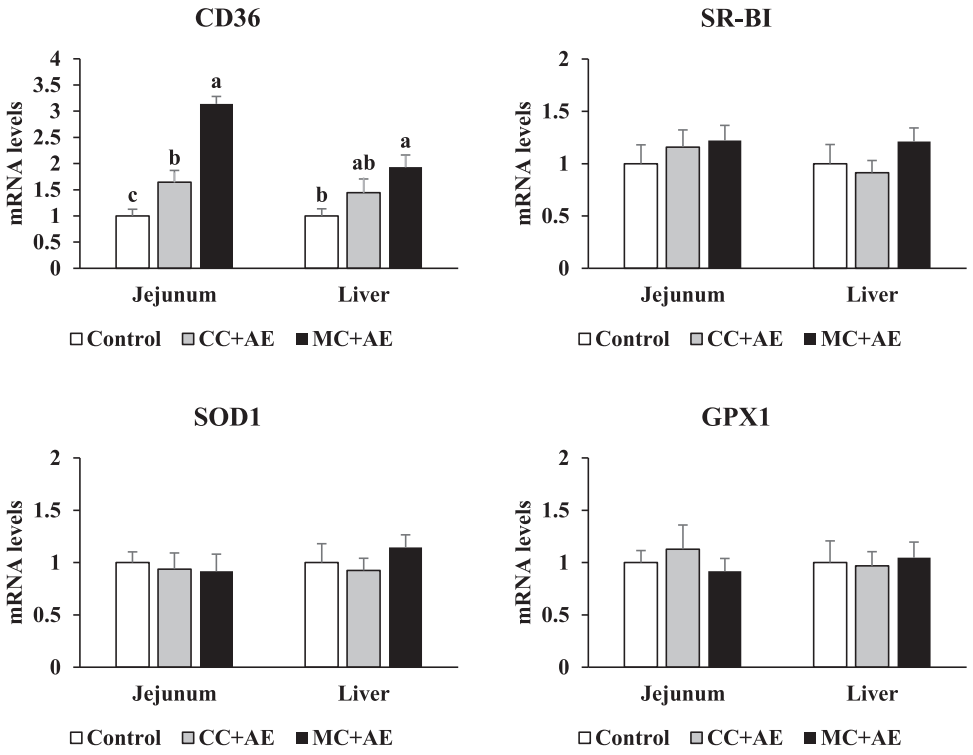

Figure 1. The mRNA expression of cluster determinant 36 (CD36), scavenger receptor class B type I (SR-BI), superoxide dismutase 1 (SOD1) and glutathione peroxidase 1 (GPX1) in jejunum and liver of laying hens (n = 6 replicates). Bars marked with different letters indicate significant different at $P < 0.05$. CC+AE, common canthaxanthin and apo-ester at 5 mg/kg each; MC+AE, microencapsulated canthaxanthin and apo-ester at 5 mg/kg each.

SOD1 and GPX1 in tissues were not affected by dietary treatments.

DISCUSSION

This study showed that dietary treatments did not affect productive performance of laying hens, although a numerical increase was found in laying rate, egg weight and egg mass, agreeing with previous studies using canthaxanthin and AE in combination (Anderson et al., 2008; Zahroojian et al., 2011). Similarly, a meta-analysis showed that canthaxanthin resulted in a numerical increase in egg production and a numerical decrease in feed conversion ratio (Umar Faruk et al., 2018). However, in this meta-analysis canthaxanthin significantly increased egg weight and egg mass, which was also observed in other studies (Englmaierová et al., 2013; Damaziak et al., 2018), implying that a beneficial effect of carotenoids on the health of hens might be involved. A recent study showed that canthaxanthin supplementation at 6 mg/kg feed significantly increased laying rate and egg weight of hens, promoted the ovulation process and maintained the reproductive hormones by improving antioxidant capacity in serum and ovaries (Zhao et al., 2023). It is difficult to explain the differences between the results obtained in our study and findings of other authors. The reasons might be, at least in part, due to the age of laying hens. It is interesting to note that peak-phase laying hens (less than 28 wk of age) were selected in the above studies with significant effects of canthaxanthin (Englmaierová et al., 2013; Damaziak et al., 2018; Zhao et al., 2023), whereas late-phase laying hens (56 wk of age) were used in our study. It could be inferred that canthaxanthin and AE supplementation had no detrimental effect on laying hens in our study. Furthermore, a tolerance study with laying hens demonstrated that canthaxanthin supplementation at 8 or 80 mg/kg feed did not affect laying performance, indicating that ten-times overdose of canthaxanthin was safe for laying hens (Weber et al., 2013). In another study, high dietary levels of AE up to 80 mg/kg feed was also reported to have no effect on productive performance of laying hens (Sirri et al., 2007).

The 2 test groups increased yolk color score of fresh eggs throughout the trial, confirming the previous observations (Anderson et al., 2008; Zahroojian et al., 2011; Basharat et al., 2023). Moreover, the MC+AE group had higher yolk color score than the CC+AE group at 24 and 36 d, suggesting that MC was more efficient. Similar results were observed in our previous study, which showed that microencapsulated lutein was better than nonmicroencapsulated lutein in yolk pigmentation (Wen et al., 2021). This finding could be explained by the time-dependent changes of yolk color in each group. Yolk color score of the CC+AE group showed a dramatic decline with time, but the MC+AE group only showed a slight decline, indicating that MC was more stable than CC. It could be confirmed by the data of feed and yolk canthaxanthin concentration in this study. Our finding was consistent with a recent study, which showed that yolk color kept steady in laying hens fed

MC supplemented diets for 4 wk (Li et al., 2024). Accordingly, the MC+AE group showed higher yolk color score in fried, boiled and stored eggs. Moreover, fried and boiled eggs had lower yolk color score than fresh eggs in each test group, indicating that frying and boiling resulted in partial degradation of carotenoids in yolk, which could be supported by the changes of yolk canthaxanthin concentration in fried and boiled eggs in this study. This finding was consistent with our previous research (Wen et al., 2021; Wen et al., 2022). No significant changes in yolk color score of stored eggs (4°C and 25°C for 20 d) indicated that carotenoids in the egg was steady during short-term storage at either cool or room temperature.

The feed canthaxanthin concentrations in the 2 test groups were similar at the beginning of the experiment, but both of them declined at the end, reflecting a possible degradation of canthaxanthin during feed storage. Similar observations have been reported in previous research (Jintasataporn and Yuangsoi, 2012), which showed that total carotenoid contents in diets decreased during storage. Heat, light and oxygen might have been the agents that most contributed to canthaxanthin degradation. The less reduction of canthaxanthin in the MC +AE group was in agreement with previous results (Hojjati et al., 2011), which showed that degradation of canthaxanthin was more retarded by microencapsulation, while canthaxanthin content of blank samples were completely deteriorated after 9 wk of storage in the light condition. Microencapsulation has been demonstrated to improve storage stability of carotenoids (Zhang et al., 2015; Zhao et al., 2018). Our finding could be supported by another study, which showed that the stability of the microencapsulated xanthophyll against light, heat and oxygen was improved by 5.6 times, 1.9 times and 7.7 times compared with nonencapsulated xanthophyll (Wang et al., 2013). The reduction of feed canthaxanthin concentration led to less canthaxanthin deposition in yolk, which was observed in the CC+AE group at 24 and 36 d. Yolk canthaxanthin concentration was reduced by frying and boiling but not storing in each test group, which was consistent with the changes of yolk color score in fried and boiled eggs. It has been reported that cooking results in some reductions in yolk color and yolk xanthophyll concentrations (Englmaierová et al., 2013; Nimalaratne et al., 2012).

No significant differences were found in the contents of TP, ALB, GLU, TG and TC in serum, implying that supplementation of carotenoids did not affect nutrient metabolism of hens. A tolerance study also showed that blood chemistry traits were not influenced by dietary canthaxanthin in laying hens (Weber et al., 2013). Higher T-AOC and lower MDA content in serum was observed in the MC+AE group, which was in agreement with previous results (Zhang et al., 2011; Zhao et al., 2023). Similar results were found in another study, which showed that a combination of canthaxanthin and AE reduced yolk MDA content in laying hens (Englmaierová et al., 2013). It has been documented that canthaxanthin has free radical-scavenging properties (Esatbeyoglu and Rimbach, 2017). No effect of CC+AE on serum MDA content might be due to dramatic reduction of canthaxanthin in feed, thus less canthaxanthin was ingested. The activities of T-SOD and GPX in serum were not affected by dietary treatments, implying that dietary canthaxanthin and AE supplementation might exhibit antioxidant activity by scavenging free radicals directly rather than altering antioxidant enzymes. Zhang et al. (2011) also found that dietary canthaxanthin did not affect serum SOD activity in breeder hens. However, canthaxanthin was shown to increase SOD and GPX activities in some other studies (Zhao et al., 2023; Li et al., 2024). It is difficult to explain the discrepancy between studies because of differences in methodological conditions, such as animal species, environment, feed composition, dosage and type of carotenoids used (Gao et al., 2013).

In this study, jejunal CD36 mRNA level was increased by the 2 test groups, suggesting that dietary supplementation of carotenoids upregulated gene expression of CD36. It has been reported that carotenoids such as lutein and astaxanthin are preferentially absorbed via a CD36-dependent mechanism (Liu et al., 2023; Moussa et al., 2011), and CD36 expression is regulated by dietary lipids (Zhao et al., 2021). Hepatic CD36 mRNA level was only increased by the MC+AE group, which might be due to improved canthaxanthin stability in MC. However, it was unclear why the mRNA expression of SR-BI was not affected. Although they both belong to the class B scavenger receptor family, they may have different expression patterns and different roles in carotenoid transport (Lobo et al., 2001; Werder et al., 2001). Very little information is currently available about the gene expression of carotenoid transporters in response to dietary intervention. No difference in SOD1 and GPX1 mRNA expression was in accordance with the data of their activities.

In conclusion, combination of canthaxanthin and AE did not affect productive performance of laying hens, but increased yolk color score of eggs, with MC being more efficient. Microencapsulation reduced canthaxanthin degradation in feed and fried and boiled eggs. In addition, supplementation of MC+AE promoted serum antioxidant capacity and CD36 mRNA expression in jejunum and liver.

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DISCLOSURES

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, 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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