The effects of canthaxanthin microencapsulation on yolk color and canthaxanthin deposition in egg yolk of laying hens

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ABSTRACT Canthaxanthin is widely used as a feed additive to improve skin and yolk color in poultry. It is insoluble in water and sensitive to oxidation, so commercial canthaxanthin is often microencapsulated with wall materials to improve its solubility and stability. The objective of this study was to evaluate the effects of canthaxanthin microencapsulation on yolk color and canthaxanthin deposition in egg yolk of laying hens. A total of 288 Hyline Brown laying hens (48 wk of age) were allocated to 4 groups with 6 replicates of 12 hens each, and fed a basal diet or the basal diet supplemented with 5 mg/kg canthaxanthin microencapsulated with modified starch (**CMMS**), gelatin (**CMG**), and sodium lignosulfonate (**CMSL**), respectively. Canthaxanthin supplementation did not affect laying performance of hens, but improved ($P < 0.05$) yolk color of fresh, fried, boiled, and stored (4 and 25°C) eggs. The improvement of yolk color of fresh eggs was greatest in the CMSL group and least in the CMG group ($P < 0.05$). Both CMMS and CMSL resulted in higher ($P < 0.05$) yolk canthaxanthin concentration than CMG. The CMSL resulted in higher ($P < 0.05$) yolk color score of fried eggs than CMMS and CMG and higher ($P < 0.05$) yolk color score of boiled eggs than CMG, but no difference was observed in stored eggs among three canthaxanthin groups. In conclusion, CMMS and CMSL were more effective in yolk pigmentation than CMG, and CMSL was slightly better than CMMS.

Key words: yolk color, laying hen, canthaxanthin, microcapsule, wall material

2022 Poultry Science 101:101889
https://doi.org/10.1016/j.psj.2022.101889

INTRODUCTION

Yolk color is an important quality trait of eggs that directly affects egg price. The preferred yolk color generally ranges from golden yellow to orange. The yolk color depends on the source and level of carotenoids in the diet (Nabi et al., 2020). Lutein and zeaxanthine color the yolk yellow, whereas canthaxanthin colors the yolk orange (Nelson and Baptist, 1968). The commercial corn-soybean meal diet for caged laying hens does not contain enough carotenoids to obtain desirable pigmentation, so exogenous pigments are usually added to the diet to improve yolk color (Lokaewmanee et al., 2011; Umar Faruk et al., 2018). The most widely used commercial carotenoid pigments in feed include lutein and canthaxanthin. All carotenoids are insoluble in water and sensitive to oxidation because of their high degree of unsaturation. To improve the bioavailability of carotenoids, various methods have been devised with the objective of enhancing their solubility and stability, in which microencapsulation technology receives increasing attention (Álvarez-Henao et al., 2018). Microencapsulation is one of promising methods for protecting unstable compounds (Gouin, 2004). It has been demonstrated that microencapsulation is able to improve the stability and bioavailability of lutein (Zhang et al., 2015; Álvarez-Henao et al., 2018; Zhao et al., 2018) and canthaxanthin (Hojjati et al., 2011, 2014). Our previous study has also shown that microencapsulated lutein is more effective in yolk pigmentation and lutein deposition than non-microencapsulated lutein in laying hens (Wen et al., 2021). However, the effect of microencapsulated canthaxanthin supplementation in laying hens has not been investigated. In addition, microencapsulation efficiency and microcapsule stability are largely dependent on the composition of wall materials, which can be selected from a wide variety of polymers such as gelatin and modified starch (Wang et al., 2012). Therefore, the objective of this study was to evaluate the effect of canthaxanthin microencapsulated with different wall materials on yolk color and canthaxanthin deposition in egg yolk of laying hens.

Received December 27, 2021.
Accepted March 22, 2022.

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MATERIALS AND METHODS

Materials

The canthaxanthin microcapsules (**CM**) containing 10% canthaxanthin were prepared by Zhejiang Medicine Co., Ltd Xinchang Pharmaceutical Factory (Shaoxing, China). Briefly, canthaxanthin was finely dispersed in the matrix of modified starch, gelatin or sodium lignosulfonate to form an emulsion by high-pressure homogenization, which was then spray-dried to form the microcapsule. The canthaxanthin microencapsulated with modified starch, gelatin and sodium lignosulfonate was designated as CMMS, CMG, and CMSL, respectively. The microcapsulation efficiency of the above microcapsules was above 95%.

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 288 Hyline Brown laying hens (48 wk of age) were used in this study. After 2 wk of adaptation to the battery cages (50 cm × 40 cm × 35 cm), the hens were allocated to 4 groups with 6 replicates of 12 hens in 4 adjacent cages (3 hens per cage). The control birds were fed a corn-soybean meal basal diet (Table 1), and the rest hens were fed the basal diet supplemented with CMMS, CMG, or CMSL at 50 mg/kg diet (5 mg/kg available canthaxanthin) for 40 d. The canthaxanthin concentrations in the 4 groups, was analyzed by high performance liquid chromatography as described in the determination of yolk canthaxanthin concentration, were 0, 5.5, 5.2, 5.7 mg/kg, respectively. 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 was calculated.

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

Item	Content
Ingredient	
Corn	630
Soybean meal (44.2% CP)	240
Limestone	100
Dicalcium phosphate	12
Methionine	1
Sodium chloride	3
Premix[1]	14
Total	1,000
Calculated nutrient composition	
Metabolizable energy (MJ/kg)	10.9
Crude protein	156.5
Lysine	7.9
Methionine	3.5
Methionine+cystine	6.1
Calcium	39.5
Available phosphorus	2.7

[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

During the experimental period, 3 fresh eggs per replicate were randomly collected for yolk color assay every 5 d. At 5, 20, 40 d of the experiment, 1 egg per replicate was randomly collected for the assay of yolk canthaxanthin concentration. At 39 d of the experiment, 8 eggs per replicate were randomly collected for the assay of yolk color of fried, boiled and stored (4°C and 25°C) eggs (2 eggs per replicate, per treatment, for each kind of cooking or storage period).

Yolk Color Assay

Yolk color of fresh eggs was analyzed by an egg multitester (EMT-7300, Robotmation Co., Ltd., Tokyo, Japan). An electric skillet (JD30AQ07, Zhejiang Supor Co. Ltd., Shaoxing, China) was used to fry eggs. Some soybean oil was poured into the frying pan and preheated, and then eggs were broken into the frying pan and fried for 1 min on each side. Then yolk was separated and yolk color was evaluated by 2 individuals independently using a DSM yolk color fan, which is consisted of 16 blades with number 1 to 16 (higher values denote more intense color), and average value was obtained. A 2,200-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 pot 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 score was evaluated by two individuals using the DSM yolk color fan. Two eggs per replicate were stored at 4°C in a refrigerator or at 25°C in an incubator for 30 d. Then the yolk color of eggs was analyzed by the egg multi-tester.

Determination of Yolk Canthaxanthin Concentration

Canthaxanthin concentration in fresh yolk (6 samples per treatment) was measured by high performance liquid chromatography (LC-20AT, Shimadzu, Tokyo, Japan). Briefly, 1 g yolk per egg 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.

Statistical Analysis

The data of laying performance and yolk color of cooked and stored eggs were analyzed by one-way ANOVA with dietary treatment as fixed effect using SPSS 22.0 software (SPSS Inc., Chicago, IL). Time effect and its interaction by diet were also included in the statistical analysis of yolk color and canthaxanthin concentration in fresh eggs using the general linear model procedure of the SPSS software. The differences among treatments were examined by Duncan's multiple range test, which were considered to be significant at $P < 0.05$. Data were presented as means and standard error of means.

RESULTS

Laying Performance

There was no difference in laying rate, egg weight, feed intake or feed conversion ratio among the groups ($P > 0.05$, Table 2). Mortality was low and showed no significant difference among groups (Data not shown).

Table 2. Effects of dietary canthaxanthin, microencapsulated with different wall materials, on performance of laying hens (n = 6 replicates).[1]

Item	Control	CMMS	CMG	CMSL	SEM	*P*-value
Laying rate, %	86.21	85.10	85.29	86.32	0.65	0.890
Egg weight, g	63.74	63.24	63.98	64.56	0.28	0.402
Feed intake, g/d	133.60	131.55	132.45	132.34	0.88	0.901
Feed conversion ratio	2.43	2.45	2.43	2.38	0.02	0.729

[1]Abbreviations: CMG, canthaxanthin microencapsulated with gelatin; CMMS, canthaxanthin microencapsulated with modified starch; CMSL, canthaxanthin microencapsulated with sodium lignosulfonate.

Yolk Color of Fresh Eggs

The yolk color of fresh eggs was affected by both diet and time, but their interaction was not significant (Table 3). Compared with the control group, CM supplementation improved ($P < 0.05$) yolk color throughout the experiment, with the CMSL group showing the greatest improvement and the CMG group showing the least ($P < 0.05$). The yolk color score of CMSL group was higher ($P < 0.05$) than that of CMG group at 5, 10, 30, and 40 d and higher ($P < 0.05$) than that of CMMS group at 40 d. The CMMS group had higher ($P < 0.05$) yolk color score than the CMG group at 20 and 40 d. The yolk color score increased ($P < 0.05$) from 5 to 15 d, and then decreased ($P < 0.05$) during 20 to 30 d, and finally increased ($P < 0.05$) from 30 to 40 d.

Yolk Canthaxanthin Concentration in Fresh Eggs

Canthaxanthin was not detected in the yolk of the control group. For the rest groups, both diet and time affected yolk canthaxanthin concentration in fresh eggs, but their interaction was not significant (Table 4). There were no significant differences in the yolk canthaxanthin concentration among three CM groups at 5 d. Compared with CMG, yolk canthaxanthin concentration was increased ($P < 0.05$) by CMMS and CMSL at 20 d and by CMSL at 40 d, and an increasing trend ($P < 0.1$) was also observed for CMMS at 40 d. The main effect of diet showed that CMMS and CMSL resulted in similar yolk canthaxanthin concentration, which was higher ($P < 0.05$) than that obtained by CMG. The yolk canthaxanthin concentration at 20 and 40 d was higher ($P < 0.05$) than at 5 d.

Table 3. Effects of dietary canthaxanthin, microencapsulated with different wall materials, on yolk color of fresh eggs in laying hens (n = 18 eggs).[1]

Duration of supplementation (d)	Yolk color score[2]				Average	SEM
	Control	CMMS	CMG	CMSL		
5	4.82^{c}	11.55^{ab}	11.16^{b}	11.97^{a}	9.87^{z}	0.37
10	6.09^{c}	12.46^{ab}	12.26^{b}	13.03^{a}	10.96^{wx}	0.35
15	6.11^{b}	12.74^{a}	12.29^{a}	12.74^{a}	10.97^{wx}	0.35
20	4.94^{c}	12.38^{a}	11.72^{b}	12.27^{ab}	10.33^{y}	0.38
25	5.24^{b}	11.99^{a}	11.39^{a}	11.97^{a}	10.15^{yz}	0.35
30	5.42^{c}	12.06^{ab}	11.41^{b}	12.32^{a}	10.30^{y}	0.32
35	6.09^{b}	12.04^{a}	12.09^{a}	12.63^{a}	10.71^{x}	0.35
40	6.77^{d}	12.72^{b}	11.91^{c}	13.43^{a}	11.21^{w}	0.32
Average	5.69^{d}	12.24^{b}	11.78^{c}	12.54^{a}		0.08
SEM	0.09	0.10	0.08	0.08	0.11	
	---------- *P*-value ----------					
Diet	<0.001					
Time	<0.001					
Interaction	0.142					

[a-d]Means within a row with different superscripts differ significantly at $P < 0.05$.
[w-z]Means within a column with different superscripts differ significantly at $P < 0.05$.
[1]Abbreviations: CMG, canthaxanthin microencapsulated with gelatin; CMMS, canthaxanthin microencapsulated with modified starch; CMSL, canthaxanthin microencapsulated with sodium lignosulfonate.
[2]Yolk color of fresh eggs was measured by an egg multi-tester.

Table 4. Effects of dietary canthaxanthin, microencapsulated with different wall materials, on yolk canthaxanthin concentration in fresh eggs of laying hens (mg/kg, n = 6 eggs).[1,2]

Duration of supplementation (d)	CMMS	CMG	CMSL	Average	SEM
5	8.17	8.53	8.73	8.48[y]	1.30
20	21.80[a]	15.53[b]	20.97[a]	19.43[x]	1.06
40	18.33[ab]	14.50[b]	22.87[a]	18.57[x]	1.39
Average	16.10[a]	12.86[b]	17.52[a]		1.01
SEM	2.12	1.33	2.51	1.01	
			P-value		
Diet	0.013				
Time	<0.001				
Interaction	0.144				

[a,b]Means within a row with different superscripts differ significantly at $P < 0.05$.
[x,y]Means within a column with different superscripts differ significantly at $P < 0.05$.
[1]Canthaxanthin was not detected in the yolk of the control group.
[2]Abbreviations: CMG, canthaxanthin microencapsulated with gelatin; CMMS, canthaxanthin microencapsulated with modified starch; CMSL, canthaxanthin microencapsulated with sodium lignosulfonate.

Yolk Color of Cooked and Stored Eggs

The yolk color of fried and boiled eggs was improved ($P < 0.05$) by all forms of CM (Table 5). The CMSL resulted in higher ($P < 0.05$) yolk color score of fried eggs than CMMS and CMG, and it also resulted in higher ($P < 0.05$) yolk color score of boiled eggs than CMG. The yolk color of stored eggs at both 4 and 25°C was improved ($P < 0.05$) in all CM groups, whereas no significant difference was observed among 3 CM groups.

DISCUSSION

The data showed that dietary supplementation of CM did not affect production performance of laying hens. Our data was consistent with the results of Rosa et al. (2012), Cho et al. (2013), and Weber et al. (2013), who found no significant effects of dietary canthaxanthin inclusion on laying rate of broiler breeders and laying hens. However, Damaziak et al. (2018) reported that dietary inclusion of canthaxanthin improved laying rate, egg weight, and feed conversion ratio of laying hens fed diets containing 1 mg/kg of iodine. A meta-analysis involving 34 trials also showed that canthaxanthin inclusion resulted in dose-dependent increases in egg mass, egg weight and feed intake, which might be due to the antioxidant effect, enhanced reproduction, and immune-modulation in response to canthaxanthin inclusion (Umar Faruk et al., 2018). The discrepancy may be attributed to the differences of diet type and experiment duration between the results obtained in our study and findings of other authors.

Table 5. Effects of dietary canthaxanthin, microencapsulated with different wall materials, on yolk color of cooked and stored eggs in laying hens (n = 12 eggs).[1]

Item	Yolk color score[2]				SEM	*P*-value
	Control	CMMS	CMG	CMSL		
Fried egg	6.33[c]	9.33[b]	7.96[b]	10.88[a]	0.35	<0.001
Boiled egg	4.54[c]	7.75[b]	6.54[b]	9.04[a]	0.36	<0.001
Stored egg at 4°C	7.43[b]	13.76[a]	13.51[a]	14.13[a]	0.43	<0.001
Stored egg at 25°C	5.97[b]	12.13[a]	12.72[a]	12.84[a]	0.45	<0.001

[a-b]Means within a row with different superscripts differ significantly at $P < 0.05$.
[1]Abbreviations: CMG, canthaxanthin microencapsulated with gelatin; CMMS, canthaxanthin microencapsulated with modified starch; CMSL, canthaxanthin microencapsulated with sodium lignosulfonate.
[2]Yolk color of fried and boiled eggs was evaluated by two individuals using the DSM yolk color fan, and yolk color of stored eggs was measured by an egg multi-tester.

All forms of CM improved yolk color of fresh eggs, agreeing with the results of previous studies (Cho et al., 2013; Sandeski et al., 2014). Compared with CMG, CMSL resulted in higher yolk color score at 5, 10, 30, and 40 d, and the same increase was induced by CMMS at 20 and 40 d, indicating that CMSL and CMMS were more effective in yolk pigmentation than CMG. The CMSL group had higher yolk color score than the CMMS group at 40 d, suggesting that the long-term effect of CMSL was better than that of CMMS. The main effect of diet also showed that CMSL and CMMS resulted in higher yolk color score than CMG. Our finding implied that sodium lignosulfonate and modified starch resulted in higher canthaxanthin bioavailability than gelatin. This may be explained by the fact that sodium lignosulfonate and modified starch have higher water-solubility than gelatin (Jane, 1992; Foo et al., 2013; Piombino et al., 2020), so the canthaxanthin in CMSL and CMMS is more finely dispersed in water and easier to be absorbed in the digestive tract. The main effect of time showed that the yolk color of fresh eggs slightly fluctuated for all groups during the experiment, which is difficult to explain.

The main effect of diet showed that CMMS and CMSL resulted in higher yolk canthaxanthin concentration than CMG, demonstrating their superior bioavailability. The yolk canthaxanthin concentration at 5 d was lower than that at 20 and 40 d, and no significant difference was observed among dietary treatments at 5 d, implying that the superiority of CMSL and CMMS was not obvious at the beginning of the experiment, when canthaxanthin deposition in yolk was relatively low. A similar pattern was observed in our previous study (Wen et al., 2021), which showed that yolk lutein concentration was lowest at 5 d, when no significant difference was observed between non-microencapsulated and microencapsulated lutein.

In order to evaluate yolk pigmentation stability, the yolk color of cooked and stored eggs was analyzed in this study. The fried and boiled eggs had lower yolk color score than fresh eggs, which is related to the denaturation of yolk protein (Llave et al., 2018). In addition, high temperature of cooking resulted in partial loss of pigments in yolk. This has been demonstrated by Nimalaratne et al (2012), who reported that cooking resulted in partial losses of lutein and canthaxanthin in egg yolk. Our previous study also showed that yolk color score and lutein content in fried and boiled eggs were lower than those in fresh eggs (Wen et al., 2021). The reduction of boiled eggs was greater than that of fried eggs, which might be due to longer time of heating during boiling. It has been reported that boiling resulted in greater reduction of egg yolk xanthophyll content than frying (Nimalaratne et al., 2012). The yolk color score of fried and boiled eggs in 3 CM groups was higher than that in the control group, indicating that they were still effective in yolk pigmentation in cooked eggs. The CMSL group had higher yolk color score of fried and boiled eggs than the CMG group, demonstrating the superiority of CMSL to CMG. It can be explained by the data of yolk canthaxanthin concentration, which was higher in the CMSL group than in the CMG group. The yolk color score of fried eggs in the CMSL group was higher than that in the CMMS group, indicating that CMSL was slightly better than CMMS, which was in parallel with the data of fresh yolk color at 40 d. The yolk color score of stored eggs in the 3 CM groups was still significantly higher than that of the control group, and it seemed that the yolk color score of stored eggs was similar to fresh eggs, indicating that yolk pigmentation was stable during storage in refrigerator or at room temperature.

In conclusion, this study indicated that dietary CM supplementation did not affect laying performance of hens, but improved yolk color of fresh, fried, boiled, and stored eggs, and CMSL and CMMS were more effective than CMG. In addition, CMSL was slightly better than CMMS according to the data of yolk color of fresh eggs at 40 d and yolk color of fried eggs.

ACKNOWLEDGMENTS

This study was funded by Jiangsu Agriculture Research System (SXGC[2017]288). We wish to thank the labmates for their technical assistance.

DISCLOSURES

The authors declare no conflict of interest.

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