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Dietary Supplementation with Microencapsulated Lutein Improves Yolk Color and Lutein Content in Fresh and Cooked Eggs of Laying Hens

Chao Wen[1], Yue Su[1], Zhengguo Tao[2], Zongjia Cheng[2], Di Zhou[3], Tian Wang[1] and Yanmin Zhou[1]

[1] College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China
[2] Guangzhou Leader Bio-technology Co. Ltd, Guangzhou 510663, China
[3] Zhejiang Medicine Co. Ltd, Xinchang Pharmaceutical Factory, Shaoxing 312500, China

This study was conducted to compare the efficacy of diet supplemented with non-microencapsulated lutein (NL) and microencapsulated lutein (ML) in laying hens. A total of 270 Hy-line Brown laying hens (54 weeks old) were allocated to three groups with six replicates of 15 hens and were adapted to a wheat-soybean meal basal diet for two weeks. Next, the control birds were fed the basal diet, and the test birds were fed the basal diet supplemented with 600 mg/kg NL (12 mg/kg available lutein) or 90.1 mg/kg ML (10 mg/kg available lutein) for 35 days. Supplementation of lutein did not affect the productive performance of laying hens, but improved ($P<0.05$) the yolk color and red/green value (a*), with eggs from the ML group displaying improved color and a* values from the 15th day of the experimental period. The blue/yellow value (b*) for the yolk showed an increase ($P<0.05$) through both NL and ML supplements. The yolk color of fried and boiled eggs and a* value of the yolk in fried eggs were improved ($P<0.05$) only through ML supplemented diet. Both NL and ML supplements resulted in lower ($P<0.05$) lightness and higher ($P<0.05$) a* values of yolk in boiled eggs, as well as higher ($P<0.05$) b* values in fried and boiled eggs. Yolk lutein content in fresh, fried, and boiled eggs was increased ($P<0.05$) in NL and ML groups with the latter being higher. In conclusion, ML improved yolk pigmentation and lutein retention in laying hens better than NL.

Key words: laying hen, lutein, microencapsulation, yolk color

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Introduction

Yolk color is an important characteristic of egg quality, and consumers generally prefer yolk colors ranging from golden yellow to orange. Yolk pigmentation results primarily from carotenoid pigments, particularly lutein, which has been known as an important antioxidant for eye health (Jang *et al*., 2014). Layers cannot synthesize lutein, and instead obtain it from their diet. However, most of the commercial poultry farms use high energy feed with low levels of lutein, therefore high levels of pigments are usually added to the diet of commercial layers to achieve the desired yolk color (Leeson and Caston, 2004). Marigold flowers (*Tagetes erecta*) are an excellent source of natural lutein and are used to improve yolk color and carotenoid content of eggs (Grčević *et al*., 2019; Titcomb *et al*., 2019). Several studies have shown that dietary supplementation of lutein extracted from marigold flowers enhances yolk color in laying hens (Chowdhury *et al*., 2008; Lokaewmanee *et al*., 2011; Skřivan *et al*., 2015). However, lutein is insoluble in water, heat-labile, and also unstable in the presence of light and oxygen because of its high degree of unsaturation, which limits its application in food and feed industry (Qv *et al*., 2011).

Microencapsulation is a coating technology that has been widely used in food and feed industry for protecting sensitive compounds from external influences, improving physical properties of a material, masking unfavorable taste, and for controlling the release of core materials (Gouin, 2004; Champagne and Fustier, 2007). Extensive studies have demonstrated that microencapsulation can be used to increase chemical stability and bioavailability of lutein (Nalawade and Gajjar, 2016; Álvarez-Henao *et al*., 2018; Steiner *et al*., 2018). It has been reported that microencapsulation increases solubility and retention rates of lutein (by about 15−50% when compared to that of free lutein), as well as its stability against heat, light, and oxygen (Wang *et al*., 2012). An *in vitro* experiment showed that lutein-loaded microcap-

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Correspondence: Dr. Yanmin Zhou, College of Animal Science and Technology, No. 1 Weigang, Nanjing, Jiangsu 210095, China.
(E-mail: zhouym6308@163.com)

sules were more easily absorbed by the intestinal Caco-2 cells than natural lutein (Zhao *et al*., 2018). A bioavailability study using Sprague-Dawley rats showed that the relative bioavailability of microencapsulated lutein (ML) was 139.1 % in comparison to a commercial reference sample (Zhang *et al*., 2015). However, few studies have investigated the efficacy of ML in poultry. The objective of this study was to evaluate the effects of ML supplement in laying hens.

Materials and Methods

Experimental Design, Diets, and Husbandry

All procedures were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee (Certification No.: SYXK (Su)2017−0007).

The non-microencapsulated lutein (NL) extracted from marigold flowers was provided by Leader Bio-technology Co. Ltd. (Guangzhou, Guangdong, China) and contained 2% lutein in powder form (the carriers were calcium carbonate and silicon dioxide). The ML containing 11.1% lutein was provided by Zhejiang Medicine Co. Ltd., Xinchang Pharmaceutical Factory (Shaoxing, Zhejiang, China) and prepared as described in Zhang *et al*. (2015). Briefly, lutein was finely dispersed in the matrix of gelatin and sucrose to form an emulsion and was then coated with corn starch. The microencapsulation efficiency was above 95%.

A total of 270 Hy-line Brown laying hens (54 weeks old) were used in this study. After two weeks of adaptation to a wheat-soybean meal basal diet (Table 1), the hens were divided into three groups with six replicates of 15 hens. The control birds were fed the basal diet, and the test birds were fed the basal diet supplemented with 600 mg/kg NL (12 mg/kg available lutein) or 90.1 mg/kg ML (10 mg/kg available lutein) for 35 days. The dietary lutein contents in the control, NL, and ML groups were estimated by high performance liquid chromatography (HPLC, LC-20AT, Shimadzu, Tokyo, Japan), which were 0.46 mg/kg, 10.61 mg/kg, and 9.50 mg/kg, respectively. Hens were allowed free access to mash feed and water throughout the experiment and were exposed to a 16:8 light-dark cycle. Rate of egg production and egg weight were recorded daily, and feed consumption was recorded weekly for every replicate. Egg mass and feed conversion ratios were calculated.

Table 1. **Ingredients and nutrient composition of the basal diet (g/kg unless otherwise stated)**

Ingredient	Content
Wheat	698
Soybean meal	150
Soybean oil	20
Limestone	80
L-lysine	2
Premix[1]	50
Total	1000
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 5th, 15th, 25th, and 35th day of the experimental period, one egg from each replicate was randomly selected, and its yolk color, albumen height, and Haugh unit were analyzed by an egg multi-tester (EMT-7300, Robotmation Co. Ltd., Tokyo, Japan). Yolk color was also measured with a colorimeter (Minolta CR-10, Konica Minolta Inc., Tokyo, Japan) using the CIELAB system (L*, a*, b*), where L* denotes lightness, a* denotes the red/green value (with green being negative and red positive) and b* denotes the blue/yellow value (with blue being negative and yellow positive). Then yolk was separated for lutein analysis. On the 35th day of the experiment, another two eggs from each replicate were randomly selected for fried and boiled egg analyses.

Yolk Color of Fried and Boiled Eggs

A 350 W electric egg cooker (JDQ-C3011, Guangdong Bears Electric Co. Ltd., Foshan, China) was used for frying eggs. Soybean oil (10 mL per egg) was poured into the frying pan (154 mm in diameter) and preheated. An egg was broken into the frying pan, which was then covered with the lid and was fried for 1 min on each side. Then yolk was separated, and yolk color was evaluated by two individuals independently using a Roche fan and the CR-10 colorimeter, and average value was determined. A 2200 W induction cooker (C22-WT2203, Midea Group Co. Ltd., Foshan, China) coupled with a stainless-steel pot was used to boil eggs. Water was poured into the pot and heated until boiling. The eggs were immersed in the boiling water for 10 min. The boiled eggs were allowed to cool, and were later cut in half, to evaluate yolk color (as described above).

Lutein Content in Yolk

Lutein content in yolk of fresh, fried, and boiled eggs (six samples each) was measured by HPLC (LC-20AT, Shimadzu, Tokyo, Japan). Briefly, 2 g of yolk per egg was added to 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 a water bath at 56℃ for 20 min. After cooling down the samples, 30 mL of hexane and 36 mL of 10% Na_2SO_4 solution were added and the samples were placed in the dark for 2 h. Lastly, aliquots from upper phase of the solution were pipetted and used for HPLC injection. Lutein was chromatographically separated by C18 column (4.6 mm × 250 mm, 5 μm) using hexane-acetone (8:2 v/v) as the mobile phase at a flow rate of 1.5 mL/min, at the detection wave-

length of 446 nm.

Statistical Analysis

All data were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software (version 22.0, SPSS Inc., Chicago, IL, USA). The differences between treatments were examined by Duncan's multiple range test, and were considered as significant at $P<0.05$. Data are presented as means and standard error of means.

Results

Productive Performance

There was no difference in laying rate, egg weight, egg mass, average daily feed intake, and feed conversion ratio between the ML and NL groups (Table 2).

Yolk Color

Compared with the control group, both NL and ML supplement improved ($P<0.05$) yolk color throughout the experiment, and the yolk color seen in ML group was better ($P<0.05$) than that seen in NL group from the 15th day of the experimental period (Table 3). The L* value was not affected by dietary supplementation of lutein. The NL group had higher ($P<0.05$) a* value of yolk than the control group from the 15th day of the experimental period, while ML supplement resulted in higher ($P<0.05$) a* value than the other two groups consistently throughout the experiment. The b* value was increased ($P<0.05$) by both NL and ML supplement, and there was no difference between the increase caused by the two groups.

For fried and boiled eggs, the ML group had slightly better

Table 2. **Effect of non-microencapsulated lutein (NL) and microencapsulated lutein (ML) on productive performance of laying hens**

Item	Control	NL[1]	ML[2]	SEM[3]	*P* value
Laying rate (%)	81.83	83.90	83.65	0.77	0.536
Egg weight (g)	64.88	65.30	65.49	0.27	0.674
Egg mass (g)	53.06	54.79	54.79	0.50	0.309
Average daily feed intake (g)	160.37	156.74	156.79	1.50	0.580
Feed conversion ratio	2.47	2.40	2.39	0.02	0.347

[1] NL: non-microencapsulated lutein.
[2] ML: microencapsulated lutein.
[3] SEM: standard error of means ($n=6$).

Table 3. **Effect of non-microencapsulated lutein (NL) and microencapsulated lutein (ML) on yolk color of fresh eggs in laying hens**

Item[1]	Control	NL[2]	ML[3]	SEM[4]	*P* value
Yolk color					
5th day	5.02^{b}	5.88^{a}	6.17^{a}	0.18	0.015
15th day	5.53^{c}	7.28^{b}	8.83^{a}	0.35	<0.001
25th day	5.43^{c}	7.27^{b}	8.22^{a}	0.30	<0.001
35th day	5.22^{c}	7.35^{b}	8.77^{a}	0.43	<0.001
L*					
5th day	60.58	60.08	62.07	0.80	0.601
15th day	62.38	63.77	60.88	0.76	0.321
25th day	60.65	60.33	60.13	0.47	0.914
35th day	62.45	61.77	59.62	0.61	0.138
a* value					
5th day	−4.47^{b}	−4.82^{b}	−2.38^{a}	0.40	0.014
15th day	−4.63^{c}	−2.17^{b}	0.40^{a}	0.54	<0.001
25th day	−3.42^{c}	−1.55^{b}	0.68^{a}	0.46	<0.001
35th day	−2.10^{c}	0.42^{b}	1.93^{a}	0.45	<0.001
b* value					
5th day	41.85^{b}	52.27^{a}	56.37^{a}	1.79	<0.001
15th day	42.17^{b}	58.03^{a}	59.90^{a}	2.23	<0.001
25th day	39.63^{b}	54.75^{a}	55.03^{a}	1.85	<0.001
35th day	43.85^{b}	59.68^{a}	59.57^{a}	2.10	<0.001

[1] L*: lightness, a* value: red/green value, b*value: blue/yellow value.
[2] NL: non-microencapsulated lutein.
[3] ML: microencapsulated lutein.
[4] SEM: standard error of means ($n=6$).
[a–c] Means with different subscripts in the same row differ significantly ($P<0.05$).

Table 4. **Effect of non-microencapsulated lutein (NL) and microencapsulated lutein (ML) on yolk color of fried and boiled eggs in laying hens**

Item[1]	Control	NL[2]	ML[3]	SEM[4]	*P* value
Yolk color					
Fried egg	3.00[b]	3.00[b]	3.67[a]	0.13	0.041
Boiled egg	3.00[b]	3.00[b]	3.50[a]	0.09	0.022
L*					
Fried egg	72.70	71.58	73.95	1.57	0.844
Boiled egg	79.22[a]	65.80[b]	66.58[b]	1.88	0.001
a* value					
Fried egg	−4.37[b]	−3.13[ab]	−1.85[a]	0.41	0.034
Boiled egg	−3.97[c]	−1.75[b]	0.13[a]	0.51	0.001
b* value					
Fried egg	44.17[b]	57.87[a]	66.10[a]	2.76	0.001
Boiled egg	43.42[c]	53.10[b]	58.32[a]	1.72	<0.001

[1] L*: lightness, a* value: red/green value, b* value: blue/yellow value.
[2] NL: non-microencapsulated lutein.
[3] ML: microencapsulated lutein.
[4] SEM: standard error of means (n=6).
[a–c] Means with different subscripts in the same row differ significantly (P<0.05).

Table 5. **Effect of non-microencapsulated lutein (NL) and microencapsulated lutein (ML) on yolk lutein content (mg/kg) of eggs in laying hens**

Item	Control	NL[1]	ML[2]	SEM[3]	*P* value
Fresh egg					
5th day	2.00[b]	4.13[a]	4.62[a]	0.36	0.001
15th day	1.71[c]	8.41[b]	14.21[a]	1.26	<0.001
25th day	1.46[c]	9.27[b]	17.04[a]	1.60	<0.001
35th day	1.68[c]	11.09[b]	18.03[a]	1.68	<0.001
Fried egg	1.39[c]	8.34[b]	15.29[a]	1.41	<0.001
Boiled egg	1.70[c]	7.27[b]	12.47[a]	1.08	<0.001

[1] NL: non-microencapsulated lutein.
[2] ML: microencapsulated lutein.
[3] SEM: standard error of means (n=6).
[a–c] Means with different subscripts in the same row differ significantly (P<0.05).

Table 6. **Effect of non-microencapsulated lutein (NL) and microencapsulated lutein (ML) on albumen height and Haugh unit of laying hens**

Item	Control	NL[1]	ML[2]	SEM[3]	*P* value
Albumen height (mm)					
5th day	6.43	7.00	6.37	0.27	0.595
15th day	6.60	6.75	6.78	3.54	0.949
25th day	6.33	6.97	6.53	2.38	0.639
35th day	6.55	6.67	6.47	0.27	0.959
Haugh unit					
5th day	77.80	78.70	72.90	2.50	0.624
15th day	78.62	80.20	80.25	1.60	0.905
25th day	77.28	81.27	79.48	1.68	0.652
35th day	79.80	76.27	78.97	1.82	0.735

[1] NL: non-microencapsulated lutein.
[2] ML: microencapsulated lutein.
[3] SEM: standard error of means (n=6).

($P<0.05$) yolk color than the control group, but no such difference was observed for the NL group (Table 4). Both NL and ML supplement resulted in lower ($P<0.05$) L* value and higher ($P<0.05$) a* value of yolk in boiled eggs, but only ML supplement increased ($P<0.05$) a* value of yolk in fried eggs. The b* value of yolk in fried and boiled eggs was increased ($P<0.05$) by both NL and ML supplement, but the effect of ML was more significant than that of NL in boiled eggs.

Lutein Content in Yolk

An increase ($P<0.05$) in the yolk lutein content in fresh eggs was observed in both NL and ML groups throughout the experiment, with the latter being higher from the 15th day of the experimental period (Table 5). The yolk lutein contents in fried and boiled eggs showed an increase ($P<0.05$) in NL and ML groups, which was higher in the latter ($P<0.05$) than the former.

Albumen Height and Haugh Unit of Eggs

Dietary lutein supplementation did not affect albumen height and Haugh unit of fresh eggs throughout the duration of our experiment (Table 6).

Discussion

This study confirmed that lutein supplementation did not affect performance of laying hens, but improved yolk color. Our finding is consistent with the data reported by Leeson *et al*. (2007) and Jang *et al*. (2014), who state that dietary lutein does not affect feed intake or egg weight, but improves yolk color of the egg. The a* and b* values of yolk were increased by dietary lutein. These observations are in agreement with studies by Santos-Bocanegra *et al*. (2004) and Titcomb *et al*. (2019). This can be explained by the fact that pigments obtained from the diet are responsible for the color of yolk, as hens cannot synthesize these pigments on their own (Skřivan *et al*., 2015). No difference observed in L* values between control group and lutein supplemented groups could be due to small amounts of lutein added in the diet. It has been reported that high levels of lutein (250 mg/kg) decrease L* value of yolk in laying hens (Englmaierová *et al*., 2013), whereas low levels of lutein (10 to 40 mg/kg) do not affect the L* value (Lokaewmanee *et al*., 2011). The ML group showed better yolk color and higher a* value than those seen in the NL group, indicating that ML was more effective than NL, which was likely due to higher bioavailability and stability of ML (Qv *et al*., 2011; Wang *et al*., 2013). Similar b* values for the two groups indicates that it may not be a good indicator to gauge the efficacy of different forms of lutein.

The yolk color of fried and boiled eggs and a* value of yolk in fried eggs were improved by ML but not NL supplement, and the b* value of yolk in boiled eggs was also higher for ML group, indicating that the cooked eggs from the ML group had better yolk color than those from the NL group. The improvement by ML supplement can be attributed to more lutein retention in yolk. Although NL supplement had no effect on yolk color in fried and boiled eggs, it resulted in lower L* value and higher a* value of yolk in boiled eggs as well as higher b* value of yolk in fried and boiled eggs. Since yolk color evaluation with Roche fan is subjective, and measurements by colorimeter are objective and more accurate, we conclude that NL supplement was able to improve yolk red/green value of boiled eggs and yolk blue/yellow value of fried and boiled eggs. It has been reported that lutein from marigold flower meal and marigold flower extract improves yolk color in raw and boiled eggs (Lokaewmanee *et al*., 2011). Failure of NL supplement in affecting L* or a* values of fried egg yolk could be attributed to higher temperature of frying, although this hypothesis requires further investigation.

The lutein content in fresh egg yolk was increased by both NL and ML supplements, with the latter being higher from the 15th day of our experiments. These observations are in agreement with the previously reported data about yolk color, and demonstrate that ML is more effective than NL and the difference between the effectiveness of the two supplements is visible after a few days. The yolk lutein contents in fried and boiled eggs were increased by NL and ML supplements, which could be attributed to the higher lutein content of fresh eggs in NL and ML groups. Fried and boiled eggs showed slightly lower yolk lutein content than fresh eggs in NL and ML group, implying that the lutein in yolk was partially damaged by heat treatment.

No significant difference in albumen height and Haugh unit of eggs between the two groups indicates that lutein supplementation does not affect egg albumen quality, which is in agreement with previously reported studies (Englmaierová *et al*., 2013; Grčević *et al*., 2019).

In conclusion, dietary lutein supplementation improved yolk color of fresh eggs, with ML group displaying higher values from the 15th day of the experimental period, but only ML supplement improved yolk color of fried and boiled eggs. Moreover, ML was more effective in increasing yolk lutein contents in fresh, fried, and boiled eggs than NL.

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Conflicts of Interest

The authors declare no conflict of interest.

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