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ARTICLE

The Tissue Distribution of Lutein in Laying Hens Fed Lutein Fortified Chlorella and Production of Chicken Eggs Enriched with Lutein

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Abstract

Two experiments were conducted to investigate the dietary effects of conventional or lutein fortified chlorella on lutein absorptions, the tissue distributions and the changes in lutein content of eggs in laying hens. In Exp 1, a total of one hundred and fifty, 70 wk-old Hy-Line brown layers were divided into three groups with five replicates and fed with each experiment diet (control diet, diet with 1% conventional chlorella or lutein fortified chlorella) for 2 wk, respectively. The egg production in groups fed diets containing both chlorella powders were higher than that of the control group (p<0.01). With chlorella supplementations, the yolk color significantly increased, although there were no significant differences in the eggshell qualities. The lutein contents of serum, liver and growing oocytes were greatly increased by feeding conventional or lutein fortified chlorella (p<0.01). In Exp. 2, a total of ninety 60 wk-old Hy-Line brown layers were assigned into three groups with three replicates per group (10 birds per replicate). The birds were fed with one of three experimental diets (0, 0.1 or 0.2% lutein fortified chlorella) for 2 wk, respectively. The egg production was not affected by dietary treatments. The egg weight in the group fed with diet containing 0.2% of lutein fortified chlorella was higher than that of the control (p<0.05). As the dietary chlorella levels increased, the daily egg mass linearly increased, although not significantly. The yolk colors in groups fed diets containing lutein fortified chlorella were dramatically increased as compared to the control (p<0.001). The lutein in chicken eggs significantly increased when fed with 0.2% of lutein fortified chlorella (p<0.01). These results suggested that the dietary lutein derived from chlorella was readily absorbed into the serum and absorbed by the liver with growing oocyte for commercial laying hens. Particularly, the lutein fortified chlorella was a valuable natural source for the production of lutein enriched chicken eggs.

Key words: conventional chlorella, lutein-fortified chlorella, egg production, lutein contents, laying hens

Introduction

Carotenoids have been used for many years in the poultry industry as a means of pigmenting eggs and meat (Leeson and Summers, 1997). Their spectral qualities result in change in color of fat depots; depending on the actual xanthophylls pigments and the concentration in the birds' diet, they impart colors ranging from yellow to the intense orange. Over the last 10 years there has been increased awareness of the role of xanthophylls in human health, and in particular the roles of lutein and zeaxanthin in prevention of certain eye disorders (Leeson and Caston, 2004). In addition, lutein, an oxygenated carotenoid, is important nutrient for the prevention of cardiovascular disease and lung cancer (Park *et al.*, 1998). Olmedilla *et al.* (2003) also suggested that lutein has been shown to reduce aged-macular degeneration and cataracts.

Chicken eggs are naturally a functional food providing various nutrients, from high quality protein to considerable levels of vitamins and other healthful compounds (Yamamoto *et al.*, 1997), of which some have health beneficial functions that are currently being studied, such as caroteinoids, lutein and zeaxanthin in egg yolk (Steinberg *et al.*, 2000). Leeson and Caston (2004) reported that the lutein content of egg yolk can be further increased by adding laying hen diet with synthetic lutein. Chlorella which is a genus of single-cell green algae provides all of

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essential nutrients and lutein has been shown to have good effects, such as immunomodulatory activity (Kotrbacek *et al.*, 1994) and intestinal microbial diversity (Janczyk *et al.*, 2009; Zheng *et al.*, 2012) in poultry. Previously, we found that the lutein greatly increased in eggs from hens fed the test diet with high levels of chlorella powder following 2 wk of dietary treatments (Jeon *et al.*, 2012). Thus, the present study was designed to determine the effect of short-term supplementation of conventional or lutein-fortified chlorella on egg qualities, tissue distribution and the changes of the lutein contents in chicken eggs.

Materials and Methods

Animals, diets and management

The conventional chlorella powder produced by *Chlorella vulgaris* were obtained from Daesang corp. The lutein fortified chlorella was produced by modified fermentation method that changed the ratio of culture media. The conventional or lutein fortified chlorella contained 1.34 mg/g or 5.36 mg/g chlorella, respectively. In Exp. 1, a total of one hundred and fifty, 70 wk-old Hy-Line brown layers were divided into three groups and fed experimental diets with conventional or lutein fortified chlorella powder or diet devoid of chlorella (as Control) for 2 wk, respectively. Each chlorella powder was substituted at the expense of commercial diets at 1% levels on weight basis. The layers were randomly placed in five replicates with 10 birds each per treatment in wire cages. The experimental diets were formulated to meet or exceed the nutrient requirements of NRC (1994) as shown in Table 1. The experimental diets and water were provided for *ad libitum* intake. A room temperature of 25±3°C and a photoperiod of 16/8 h light/dark cycle were maintained throughout the experimental period. The experimental diets were freshly added everyday and the feed intake of each group was recorded weekly.

In Exp. 2, a total of ninety, 60 wk-old Hy-Line brown layers were divided into three groups and fed one of the three diets with 0 (as control), 0.1, or 0.2% lutein fortified chlorella powder for 2 wks, respectively. Lutein fortified chlorella was added at reasonable levels considering the industrial application and the price of the product. The layers were randomly placed in three replicates with 10 birds each per treatment in wire cages. The laying hens had free accessed to the experimental diets and water. A room temperature of 22±3°C and a photoperiod of 16/8 h light/dark cycle were maintained throughout the experimental period. The experimental diets were freshly added everyday and the feed intake of each group was recorded weekly. All animal care procedures were approved by Institutional Animal Care and Use Committee in Konkuk University.

Table 1. Formula and chemical composition of experimental diet (Exp. 1 and 2)

Ingredients	Diet
	---------- % ----------
Corn	52.58
Wheat	8.00
Soybean meal	13.80
Rapeseed meal	5.00
Corn glutein meal	1.50
Dried distillers grains with solubles	5.00
Limestone, coarse	9.90
Molasses	1.00
Dicalcium phosphate	1.10
Salt	0.32
Tallow	0.90
Choline-chloride, 50%	0.08
DL-Methionine, 98%	0.12
L-Lysine, 25%	0.48
Threonine, 98%	0.02
Mineral mix[1]	0.10
Vitamin mix[2]	0.10
Total	100.00
Calculated value of basal diet	
Dry matter, %	89.32
Crude protein, %	15.50
Ether extract, %	3.46
Crude fiber, %	2.70
Crude ash, %	13.23
Ca, %	4.15
Available P, %	0.34
MEn, kcal/kg	2,780

[1]Mineral mixture provided following nutrients per kg of diet: Fe, 70 mg; Mn, 8 mg; Cu, 7.5 mg; I, 1 mg; Se, 0.2 mg; Co, 0.13 mg.
[2]Vitamin mixture provided following nutrients per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,300 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3.5 mg; vitamin B_{12}, 0.02 mg; biotin, 0.12 mg; niacin, 30 mg; pantothenic acid, 10 mg; folic acid, 0.7 mg

Egg production and eggshell qualities

In Exp. 1 and 2, egg production was recorded daily by replicate (number of eggs / number of live birds×100) and the mean egg weight was determined by the daily average weight of egg, excluding abnormal eggs (soft-shell plus broken eggs). Eggshell strength, eggshell thickness, and eggshell color were measured on 30 eggs collected randomly from 6 replicates of each treatment biweekly. The eggs were weighed individually and then were exposed to a breaking force by using an eggshell strength tester (FHK,

Fugihira, Ltd, Japan). Eggshell strength was measured as the maximum force (N) required to fracture each egg. On breaking, the egg contents were poured into a glass plate to measure the albumen height. Haugh unit values, along with albumen height and egg weight, were determined using a QCM$^+$ Tester (QCM$^+$, Technical Services and Supplies Ltd., York, England). Eggshell thickness was measured with a digimatic thickness micrometer gauge (Digimatic micrometer, Series 293-330, Japan) on a piece of shell from the equatorial region. Egg yolk color was measured by comparing with Roche egg yolk color fan (Yolk color fan, Switzerland). Eggshell color was also measured using a QCM$^+$ Tester.

Analysis of lutein in serum, liver, growing oocyte and egg yolks

At the end of Exp. 1, eight birds were randomly selected from each group. Thereafter, the blood was drawn from wing vein of each bird for determination of the lutein concentration. At necropsy, the liver and growing oocyte were immediately removed and stored in the refrigerator (4°C) until use. Lutein contents in serum, liver and growing oocyte were determined according to the method of Schlatterer and Breithaupt (2006) with some modification. In brief, an aliquot of samples was placed in a round-bottom flask with 45 mL of ternary solvent mixture (light petroleum / ethyl acetate / methanol, 1:1:1, v/v/v). 2 mL of distilled water was added to the flask in order to facilitate separation. The separation was involved two immiscible liquid phases, the upper layer phase was recovered and then 2 mL of ethanol was added to remove water. After vacuum evaporation (50 mBar, 30°C for 10 min), the extract including fatty residues was transferred to the volumetric flask with TMBE/methanol (1:1, v/v) to a total of 10 mL. The extracts were filtered through a 0.45 μm filter membrane (Whatman No. 6789, England) and assayed using HPLC (Beckman counter Inc., USA). In Exp. 2, egg yolks separated from albumen were collected and thawed in the refrigerator (4°C) until use. Lutein content in egg yolk was measured to the same method in Exp. 1.

Statistical analysis

The differences in treatment effects among groups were evaluated by ANOVA using the general linear models procedure of SAS (SAS, 2005) and significant differences were determined using Duncan's multiple range test at $p<0.05$ (Duncan, 1955).

Results and Discussion

Egg production

In Exp. 1, there was no significant difference in feed intake of birds fed experimental diets as shown in Table 2. Egg production and daily egg mass in groups fed diets with conventional or lutein-fortified chlorella were significantly higher than those of control ($p<0.01$). Egg weights were not affected by dietary treatments. In Exp. 2, there was no significant difference in egg production in layers fed experimental diets as shown in Table 3. The daily egg mass in groups fed diets with chlorella powder tended to be higher than that of control group. The egg weight was the highest in the layers fed diet with 0.2% chlorella powder and the lowest in the control group ($p<0.05$) In previously, dietary chlorella powder and culture media had positive effect on laying performance (Jeon *et al.*, 2012). Zheng *et al.* (2012) also reported that fermented chlorella supplemented diets positively affected the egg production

Table 2. The dietary effects of conventional or lutein fortified chlorella powder on egg production in laying hens (Exp. 1)[1]

	Control	CC	LC	Pooled SEM	P value
Feed Intake, g/day/hen	125.9	128.9	128.5	1.69	0.10
Egg production, %	60.8^b	66.5^a	69.4^a	1.02	<0.01
Egg weight, g/egg	68.9	68.0	68.9	0.54	0.18
Daily egg mass, g/d	41.9^b	45.6^a	47.2^a	0.80	<0.01

[1]Control, basal diet; CC, basal diet+conventional chlorella powder 1%; LC, basal diet+lutein-fortified chlorella powder 1%
[a,b]Mean values with different superscripts within the same row differ significantly ($p<0.05$).

Table 3. The dietary effects of lutein fortified chlorella powder egg production in laying hens (Exp. 2)[1]

	Control	T1	T2	Pooled SEM	P value
Egg production, %	79.2	82.2	79.5	2.40	0.64
Egg weight, g/egg	63.9^b	63.8^b	67.8^a	1.25	0.05
Daily egg mass, g/d	50.6	52.4	53.9	0.75	0.49

[1]Control, basal diet; T1, basal diet+chlorella powder 0.1%; T2, basal diet+chlorella powder 0.2%
[a,b]Mean values with different superscripts within the same row differ significantly ($p<0.05$).

in aged laying hens. On the other hands, Halle *et al.* (2009) observed that there were no dietary effects of chlorella on egg production, egg weight and feed intake during early laying stage. Obviously, the conventional or lutein-fortified chlorella seemed to have some advantages in improving egg production of aged laying hens and it might partly due to additional provision of crude protein and essential nutrients. The long term feeding studies using a larger number of laying hens with different ages are suggested to clarify effects on laying performance.

Internal and external egg qualities

The dietary effects of conventional or lutein fortified chlorella on egg and eggshell qualities are shown in Table 4. Eggshell color, shell strength and thickness were not influenced by the dietary treatments. The Haugh unit was tended to be increased in the layers fed diet with both chlorella, but not significantly. The egg yolk color in groups fed diets with conventional or lutein fortified chlorella were significantly higher than that of control ($p<0.05$). The dietary effects of lutein fortified chlorella on egg and eggshell qualities are shown in Table 5. There were no significant differences in eggshell color, shell strength and thickness among groups. Haugh unit was not also influenced by the dietary treatments and it was conflicting with our previous study (Jeon *et al.*, 2012). The egg yolk color in groups fed diets with lutein fortified chlorella powder was significantly higher than that of control ($p<0.001$). Grau and Kelin (1957) and Belyavin and Marangos (1989) suggested that yolk color was elevated in layers fed diets containing microalgae rich in xanthophylls. These results are also in agreement with Leeson and Caston (2004) who found that there was a significant increase in egg yolk color as lutein was supplemented to the layer diets. The diets with 0.1 and 0.2% lutein fortified chlorella exerted egg quality-improving effects in aged laying hens.

Lutein contents of various tissues and egg yolk

The dietary effects of conventional or lutein fortified chlorella on carotenoids concentrations of liver, growing occytes and serum are shown in Table 6. The lutein contents of liver in groups fed diets with conventional or lutein-fortified chlorella were significantly increased as compared with that of control ($p<0.001$). The lutein content was higher from lutein fortified chlorella group than

Table 4. The dietary effects of conventional or lutein fortified chlorella powder on egg qualities in laying hens (Exp. 1)[1]

	Control	CC	LC	Pooled SEM	P value
Eggshell color	27.6	27.1	26.4	0.58	0.47
Yolk color, RCF	5.6[b]	6.2[a]	6.5[a]	0.10	0.03
Eggshell strength, kg/cm^2	2.5	2.4	2.3	0.09	0.40
Eggshell thickness, 0.01mm	34.2	34.1	34.3	0.43	0.39
Haugh unit	73.7	76.1	76.4	1.46	0.15

[1]Control, basal diet; CC, basal diet+conventional chlorella powder 1%; LC, basal diet+lutein-fortified chlorella powder 1%
[a,b]Mean values with different superscripts within the same row differ significantly ($p<0.05$).

Table 5. The dietary effects of lutein fortified chlorella powder on egg qualities in laying hens (Exp. 2)[1]

	Control	T1	T2	Pooled SEM	P value
Eggshell color	27.6	27.0	27.6	0.73	0.70
Yolk color, RCF	6.0[b]	10.0[a]	11.0[a]	0.15	0.001
Eggshell strength, kg/cm^2	2.7	2.8	2.8	0.43	0.30
Eggshell thickness, 0.01mm	34.7	35.3	35.5	0.11	0.77
Haugh unit	72.9	74.3	73.8	1.49	0.077

[1]Control, basal diet; T1, basal diet+chlorella powder 0.1%; T2, basal diet+chlorella powder 0.2%
[a,b]Mean values with different superscripts within the same row differ significantly ($p<0.05$).

Table 6. The dietary effects of conventional or lutein fortified chlorella powder on lutein contents of liver, growing oocytes and serum in laying hens (Exp. 1)[1]

	Control	CC	LC	Pooled SEM	P value
Liver, ug/g	2.12[c]	6.84[b]	12.4[a]	0.81	<0.001
Growing oocytes, ug/g	8.03[c]	29.37[b]	57.64[a]	6.66	<0.0004
Serum, mg/ℓ	92.09[b]	332.79[a]	442.03[a]	46.71	0.0004

[1]Control, basal diet; CC, basal diet+conventional chlorella powder 1%; LC, basal diet+lutein-fortified chlorella powder 1%
[a-c]Mean values with different superscripts within the same row differ significantly ($p<0.05$).

Table 7. The dietary effects of lutein fortified chlorella powder on the lutein content of egg yolk in laying hens (Exp. 2)[1]

	Control	T1	T2	Pooled SEM	P value
Egg yolk, mg/egg	0.65[b]	0.73[b]	0.88[a]	0.10	0.01

[1]Control, basal diet; T1, basal diet+chlorella powder 0.1%; T2, basal diet+chlorella powder 0.2%.
[a,b]Mean values with different superscripts within the same row differ significantly ($p<0.05$).

from conventional chlorella group ($p<0.001$). The lutein contents in groups fed diets with conventional or lutein-fortified chlorella were also significantly increased as compared with that of control ($p<0.001$). The lutein content was also higher from lutein fortified chlorella group than from conventional chlorella group ($p<0.001$). With feeding of both chlorella, the lutein contents of serum were greatly increased as compared with that of control (Table 6).

It has been well known that dietary lutein was readily absorbed into the blood and taken up by various tissues in humans (Parker, 1989), rodents (Chew *et al.*, 1996) and avian (Tyczkowski and Hamilton, 1986). The concentration of blood lutein had rapidly increased in mice fed diet with lutein on feeding of 3 d (Park *et al.*, 1998). In this study, the lutein contents of serum and other tissues in groups fed diets with conventional or lutein-fortified chlorella were significantly increased as compared with that of control. The level of serum lutein is a key indicator of its bioavailability from feed (Chung *et al.*, 2004). The major carotenoid in chlorella is lutein and Jeon *et al.* (2012) found that the egg lutein greatly increased with increasing levels of chlorella powder in laying hens, in previous study. It is assume that chlorella lutein was absorbed through the intestinal wall into blood stream where it was transported to the liver, a storage site, and subsequently transported to the growing oocytes. In addition, the laying hens fed diet with lutein-fortified chlorella had significantly greater contents of lutein and carotenes for all tissues compared to group fed diet with conventional chlorella. Thus feeding of lutein-fortified chlorella will be effectively used to get desirable products with an increased lutein as well as other carotenoids.

The dietary effects of lutein fortified chlorella on the lutein contents of egg yolk are shown in Table 7. The lutein contents of egg yolks in group fed diet containing 0.2% lutein fortified chlorella significantly increased as compared with that of control ($p<0.01$). It has been reported that there was an increase in the lutein contents in eggs produced by diets with pure lutein and a natural source containing lutein. Leeson and Caster (2004) found that adding various levels of lutein to the layer diets resulted in a dramatic increase in the lutein levels of egg yolks, although transfer efficiency was very low at higher levels of inclusion. Karadas *et al.* (2006) also reported that the lutein content of the egg yolk was significantly increased after feeding diet with 0.2% marigold extract in Japanese quails. In previous study, the lutein was transferred into the egg yolks increasing from a basal level of about 0.2 mg/egg (13 μg/g yolk in control group) to 0.43 mg/egg (27 μg/g yolk in group fed diet with 3% chlorella powder) (Jeon *et al.*, 2012). The eggs enriched with lutein will represent a beneficial contribution to human diet, because daily intake of lutein in adults seems to be very low (Landrum and Bone, 2001). The lutein fortified chlorella used in this study represents a valuable tool for the production of chicken eggs enriched with natural lutein.

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