Korean J. Food Sci. Ani. Resour.
Vol. 32, No. 1, pp. 13~17(2012)
DOI http://dx.do.org/10.5851/kosfa.2012.32.1.13

ARTICLE

The Production of Lutein-Enriched Eggs with Dietary Chlorella

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Abstract

Two experiments were conducted to investigate the dietary effect of *chlorella vulgaris* on egg production and lutein incorporation into chicken eggs. In Exp. 1, a total of three hundred, 70 wk-old Hy-Line brown layers were divided into six groups with five replicates and fed each experimental diet (corn-SBM based control diet and diets with 0.1, 0.3 or 0.5% chlorella powder and with 0.8 or 2.4% chlorella cultured media) for 6 wk, respectively. The egg production in the groups fed diets containing the chlorella powder and chlorella cultured media were higher than that of the control group ($p<0.001$). As dietary chlorella levels increased, the yolk color linearly increased. However, there were no significant differences in eggshell qualities. The layers fed diet with 2.4% chlorella cultured media showed the highest Haugh unit value. In Exp. 2, a total of one hundred-eight 80 wk-old Hy-Line brown layers were assigned into four groups with three replicates per group (9 birds per replicate). The birds were fed one of four experimental diets (0, 0.5, 1.0 or 2.0% chlorella powder) for 4 wk, followed by a 14 d feeding of a withdrawal diet devoid of chlorella powder. At 2 wk, the lutein greatly increased with increasing levels of chlorella powder in birds fed diets containing more than 1%. The maximum incorporation of lutein into eggs was reached after 2 or 3 wk of feeding diets with chlorella powder. After a 7 d withdrawal, the lutein contents of egg yolks in the groups fed diets with more than 1% chlorella powder were still higher than that of control group ($p<0.05$). No significant differences in the lutein levels were found among groups after a 14 d withdrawal period. These results indicated that the use of chlorella in layer diets was effective in improving egg production and egg quality and for the production of lutein fortified eggs.

Key words: chlorella powder, lutein content, egg production, egg quality, laying hens

Introduction

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). Daily intake of several carotenoids by humans has been known to be health benefit. For example, lutein, an oxygenated carotenoid found in egg yolk, has been shown to reduce aged-macular degeneration and cataracts (Olmedilla *et al*., 2003). In addition, lutein has a strong antioxidant capacity (Sindhu *et al*., 2010) and might be useful in preventing the incident of cancer (Park *et al*., 1998). A recent research has indicated that the lutein content of egg yolk can be further increased by adding laying hen feed with lutein (Leeson and Caston, 2004).

Naturally occurring lutein is found mainly in higher plants and algae. Especially chlorella which is a genus of single-cell green algae, is high in protein, polyunsaturated fatty acids, micro nutrients and provides all of dietary essential amino acids in excellent ratios. Compare with higher plants, chlorella has an advantage because it can be cultivated in bioreactors on a large scale and thus are continuous and reliable source of product. It has been known that dietary intake of chlorella is effective in lowering blood lipid (Shibata *et al*., 2001) and enhancing immune response (An *et al*., 2008). Kotrbacek *et al*. (1994) also reported that feeding diet with chlorella bio-

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mass increased the live weight and phagocytic activity of broiler chicks. But only limited information is available on the feeding types of chlorella on performance and product characteristics in other avian species. The aims of present study were to evaluate the dietary effects of chlorella power and cultured media on egg production and egg qualities and the changes of the lutein contents in chicken eggs.

Materials and Methods

Animals, diets and management

The chlorella powder and chlorella cultured media (liquid chlorella) were provided by Daesang corp. The culture media (12.5% chlorella) was commercially produced by tank fermentation. In Exp. 1, a total of three hundred, 70 wk-old Hy-Line brown layers were divided into six groups and fed one of the six diets with 0 (as control), 0.1, 0.3 or 0.5% chlorella powder and with 0.8 or 2.4% chlorella cultured media for 6 wk, respectively. 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 (NRC, 1994) as shown in Table 1. The experimental diets and water were provided for *ad libitum* intake. A room temperature of 18±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. At the end of the experiment period, 10 birds from each group were selected and weighed individually. The blood was drawn from the jugular vein using a syringe for determination of blood profiles. The serum was separated from each blood sample via centrifugation and stored at -30°C until it was used.

In Exp. 2, a total of one hundred-eight 80 wk-old Hy-Line brown layers were randomly allocated with four treatments with three replicates of 9 birds each per treatment in wire cages. The layers fed one of four experimental diets containing 0, 0.5, 1 or 2% chlorella powder for 4 wk, followed by 14 d feeding of a withdrawal diet without chlorella powder. Chlorella powder was substituted at the expense of experimental diets at 0.5, 1.0 or 2.0% levels on weight basis, respectively. The experimental diets and water were provided *ad libitum*. 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

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, 10mg; folic acid, 0.7 mg

Egg production and eggshell qualities

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., England). Eggshell thickness was measured with a digimatic thickness micrometer gauge (Digimatic micrometer, Series 293-330, Mitutoyo, 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, Roche, Switzerland). Eggshell color was also measured using a QCM$^+$ Tester.

Blood profiles

The activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were estimated according to the colorimetric method using GOT-GPT diagnostic kit (BSC GOT-GPT assay kit, Bio Clinical System Corporation), following the manufacturer's direction. The levels of creatine and blood urea nitrogen (BUN) were determined by an autoanalyzer (Hitachi 7600-110, Japan) using the enzymatic procedures.

Analysis of lutein in egg yolks

Egg yolks separated from albumen were collected weekly and stored at -20°C for the analysis of egg lutein. The egg yolks thawed in the refrigerator (4°C) until use. Lutein content in egg yolk was determined according to the method of Schlatterer and Breithaupt (2006). In brief, 4.5 g of the egg yolks 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 extracted egg yolks were filtered through a 0.45 μm filter membrane (Whatman No. 6789, England) and assayed using HPLC (Beckman Coulter Inc., USA).

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

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 chlorella powder and liquid chlorella were significantly higher than those of control ($p<0.001$). The laying performance was the highest in the layers fed diet with 0.3% chlorella powder and the lowest in the control group. Egg weights in groups fed diets with liquid chlorella were tended to be decreased as compared with those of groups fed diets with chlorella powder. An improved laying performance in birds fed diets with chlorella was conflicting with the result of experiment using relatively young layers. Halle *et al*. (2009) observed that there were no effects of dietary chlorella on egg production, egg weight and feed intake in 26 wk-old layers. On the other hand, dietary spirulina at the level of 2% had positive effect on egg production without improvement of egg weight and feed intake (Oh *et al*., 1995). Longer term studies using a larger number of laying hens with different ages are suggested in order to clarify effects on laying performance.

The dietary effects of chlorella powder and liquid media on egg quality are shown in Table 3. Eggshell color, shell strength and thickness were not influenced by the dietary treatments. The Haugh unit was the highest in the layers fed diet with 2.4% liquid chlorella and the lowest in the control group. The Haugh unit is regarded to be the most widely accepted indicator of internal egg quality (Williams, 1992). It was reported that certain natural antioxidants such as vitamin C, vitamin E and selenium being beneficial to albumen quality by their antioxidant properties (Sahin *et al*., 2003), although general nutrients in

Table 2. The dietary effects of chlorella powder and liquid media on feed intake and egg production in laying hens (*Exp*. 1)[1)]

	Control	T1	T2	T3	T4	T5	Pooled SEM	P value
Feed Intake, g/day/hen	125.9	128.9	128.5	124.2	125.2	130.8	1.69	0.10
Egg production, %	60.8^{d}	66.5^{c}	75.4^{a}	69.5^{bc}	71.4^{b}	69.1^{bc}	1.02	<0.001
Egg weight, g/egg	68.9^{a}	68.0^{ab}	68.9^{a}	67.3^{abc}	66.2^{c}	66.4^{bc}	0.54	0.001
Daily egg mass, g/d	41.9^{c}	45.6^{b}	52.2^{a}	46.9^{b}	47.3^{b}	45.3^{b}	0.80	<0.001

[1)]Control, basal diet; T1, basal diet + chlorella powder 0.1%; T2, basal diet + chlorella powder 0.3%; T3, basal diet + chlorella powder 0.5%; T4, basal diet + liquid chlorella 0.8%; T5, basal diet + liquid chlorella 2.4%

[a-d]Mean values with different superscripts within the same row differ significantly ($p<0.05$).

Table 3. The dietary effects of chlorella powder and liquid media on egg internal and external qualities in laying hens (*Exp.* 1)[1)]

	Control	T1	T2	T3	T4	T5	Pooled SEM	P value
Eggshell color	27.59	27.13	26.38	27.82	27.12	26.65	0.58	0.470
Yolk color, RCF	5.65^{c}	5.78^{bc}	6.50^{a}	6.52^{a}	5.76^{bc}	5.96^{b}	0.10	0.001
Eggshell strength, kg/cm^2	2.49	2.42	2.27	2.47	2.43	2.53	0.09	0.394
Eggshell thickness, 0.01 mm	34.18	34.08	34.28	35.01	34.59	35.17	0.43	0.390
Haugh unit	73.73^{c}	76.10^{bc}	79.41^{ab}	76.82^{bc}	78.51^{ab}	81.70^{a}	1.46	0.003

[1)]Control, basal diet; T1, basal diet + chlorella powder 0.1%; T2, basal diet + chlorella powder 0.3%; T3, basal diet + chlorella powder 0.5%; T4, basal diet + liquid chlorella 0.8%; T5, basal diet + liquid chlorella 2.4%
[a-c]Mean values with different superscripts within the same row differ significantly (p<0.05).

layer diets did not appear to have any beneficial effect on Haugh unit. The egg yolk color in groups fed diets with the chlorella powder and liquid chlorella were significantly higher than that of control, except for T4 group. The result are in agreement with those of Grau and Kelin (1957) and Belyavin and Marangos (1989) who suggested that yolk color was elevated in layers fed diets containing microalgae rich in xanthophylls. Leeson and Caston (2004) found that there was a significant increase in egg yolk color as lutein was supplemented to the layer diets. In this study, diets with 0.3 and 0.5% chlorella powder and with liquid media exerted egg quality-improving effects in aged laying hens.

The levels of various blood profiles are presented in Table 4. There were no significant differences in total protein, BUN and creatine among groups. The activities of serum GPT were not also influenced by the dietary treatments. Measurement of serum GOT and GPT activities indicative of liver or tissue damages in avian species is a valuable tool to determine a safe inclusion rate for non-conventional feedstuff and feed additives (Diaz *et al*., 2003). Chlorella powder and liquid media appeared safe at the inclusion rate of 0.5% and 2.4%, respectively, and will be recommended at this point without having adversary effect on physiological status in laying hens.

The dietary effects of chlorella powder on the lutein contents of egg yolk are shown in Table 5. At 1 wk, the lutein contents of egg yolks in groups fed diets containing chlorella powder more than 2% significantly increased as compared with that of control. At 3 wk, as the chlorella powder of the diet increased, there was an increase in lutein content of egg yolks (p<0.01). The maximum incorporation of lutein into eggs was reached after 2 or 3 wk of feeding diets with chlorella powder. The highest level of yolk enrichment was found in the groups fed diet with 2% chlorella powder. On 7 d withdrawal, the lutein contents of egg yolks in groups fed diets with more than 1% chlorella powder were still higher than that of control (p<0.01). No significant differences in the lutein levels were found among the groups after 14 d withdrawal period.

It has been reported that there was an increase in the

Table 5. The dietary effects of chlorella powder on the lutein concentration of egg yolk in laying hens (*Exp.* 2)[1)]

wk	Control	T1	T2	T3	Pooled SEM	P value
	μg/g					
1	13.55^{c}	15.42^{bc}	16.84^{b}	19.72^{a}	0.68	0.01
2	11.89^{b}	13.28^{b}	19.11^{a}	20.69^{a}	1.20	0.01
3	13.56^{d}	16.90^{c}	20.56^{b}	24.10^{a}	1.01	0.01
4	13.88^{c}	16.83^{bc}	17.82^{b}	27.04^{a}	1.19	0.01
5	11.55^{c}	12.30^{c}	14.36^{b}	16.13^{a}	0.44	0.01
6	13.34	13.70	14.18	13.31	0.79	0.80

[1)]Control, basal diet; T1, basal diet + chlorella powder 0.5%; T2, basal diet + chlorella powder 1.0%; T3, basal diet + chlorella powder 2.0
[a-d]Mean values with different superscripts within the same row differ significantly (p<0.05).

Table 4. The dietary effects of chlorella powder and liquid media on various blood profiles in laying hens (*Exp.* 1)[1),2)]

	Control	T1	T2	T3	T4	T5	Pooled SEM	P value
Total protein, g/dL	5.34	5.23	5.02	5.48	4.80	4.70	0.26	0.233
GOT, IU/L	210.80^{a}	174.17^{c}	181.17^{bc}	196.50^{ab}	204.50^{a}	190.00^{ab}	6.60	0.005
GPT, IU/L	1.43	1.17	1.86	1.86	1.67	1.40	0.27	0.397
BUN, mg/dL	1.89	2.23	2.26	2.08	2.03	1.78	0.13	0.080
Creatine, mg/dL	0.33	0.33	0.34	0.38	0.33	0.33	0.02	0.333

[1)]Control, basal diet; T1, basal diet + chlorella powder 0.1%; T2, basal diet + chlorella powder 0.3%; T3, basal diet + chlorella powder 0.5%; T4, basal diet + liquid chlorella 0.8%; T5, basal diet + liquid chlorella 2.4%
[2)]Abbreviation used: GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; BUN, blood urea nitrogen
[a-c]Mean values with different superscripts within the same row differ significantly (p<0.05).

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 this 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 2% chlorella powder).

The chicken eggs are considered to be a good source of the lutein. Eggs generally contained 0.3 to 0.5 mg of total xanthophylls with just over half present as lutein (Steinberg *et al*., 2000). It has been well known that lutein has a strong antioxidant capacity (Sindhu *et al*., 2010) and has been shown to reduce aged-macular degeneration and cataracts (Olmedilla *et al*., 2003). Lutein enrichment of chicken eggs, as shown here, will represent a beneficial contribution to human diet, because daily intake of lutein in adults seems to be very low (Landrum and Bone, 2001). For production of lutein fortified chicken eggs, it takes at least 2 or 3 wk of feeding diets with chlorella powder to ensure the maximum incorporation of lutein into eggs.

Acknowledgment

This study is supported by a grant of national R&D project of Rural Development Administration in 2011.

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(Received 2011.9.23/Revised 2011.12.13/Accepted 2011.12.14)