Effects of Dietary Lutein Sources on Lutein-Enriched Egg Production and Hepatic Antioxidant System in Laying Hens

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The present study was conducted to investigate the effects of two dietary lutein sources such as the commercial lutein and the emulsified crude extract of spinach containing lutein on the transfer of lutein into egg yolks as well as the antioxidant defense system in the liver of laying hens. A total of thirty-six, 24-week-old White leghorn hens were randomly assigned to a basal diet (CON) and that supplemented with a commercial lutein (LUT, 40 mg lutein /kg of diet) and the crude extract of spinach dissolved into oils with lecithin (ECE, 40 mg lutein/kg of diet) for 5 weeks. There was no difference in body weight and the relative live weight among dietary groups. The concentration of egg yolk lutein and yolk color significantly increased ($P<0.05$) in the LUT and ECE groups compared with the CON group. The LUT group showed a higher yolk lutein and much a lower variability of average yolk lutein content, although there was no significant difference in egg yolk lutein content between the LUT and ECE groups. In antioxidant activity, the specific activity of hepatic superoxide dismutase (SOD) in the LUT group was significantly ($P<0.05$) greater than that in the CON and ECE groups, whereas glutathione peroxidase (GPX) and glutathione S-transferase (GST) activities and lipid peroxidation were not affected by dietary sources of lutein. In conclusion, the dietary supplementation of lutein and the emulsified crude extract of spinach to laying hens resulted in a significant increase in the content of egg yolk lutein and yolk color, indicating that both supplements may potentially be applicable for the production of egg-enriched lutein in laying hens. This study also suggest that a commercial lutein more consistently produces the quality of lutein-enriched eggs and improves hepatic SOD activity compared with the emulsified crude extract of spinach.

Key words: antioxidants, crude extract of spinach, laying hens, lutein, yolk lutein


Introduction

A number of carotenoids have been extensively used for several decades in the poultry industry as a means of pig-menting eggs to improve egg quality (Leeson and Summers, 1997). Carotenoids are coloring agents that exist naturally in plant-based feedstuffs; some of these are transferred into egg yolk. Among the 600 carotenoids found in plants, lutein and zeaxanthin are major dietary oriented xanthophyll compo-nents (Ahmed et al., 2005) and serves as an antioxidant in the body (Alves-Rodrigues and Shao, 2004). Over the last decade, xanthophylls have been known as an important dietary antioxidant for eye health, particularly in the role of lutein in the prevention of age-related macular degeneration (Berendschot et al., 2000). As lutein is not synthesized in the body, dietary ingestion is the only source to meet the requirements of lutein to prevent this disease and improve the antioxidant defense system. Lutein has been known to have excellent antioxidant ability, because of its capability to quench singlet oxygen and scavenge peroxyl radicals in cellular membrane (Lim et al., 1992).

The bioavailability of lutein in humans is extremely variable mostly due to the inherent complex of the food matrix, poor solubility and the interaction of carotenoids with
other nutrients (Mamatha and Baskaran, 2011). In fact, the dietary matrix and the type of lutein are important factors that affect lutein absorption, indicating that the egg yolk is the most effective way to increase lutein bioavailability, although the amounts are relatively lower than other lutein-rich vegetables including spinach (Handelman et al., 1999; Surai et al., 2000).

It has been reported that the egg yolk normally contains the level of 0.14 to 0.16 mg of lutein (Leeson and Caston, 2004; Goodrow et al., 2006). The deposition of lutein in the normal egg yolk is markedly affected by the dietary sources, depending on the type and the concentration of lutein, extent of processing, etc. (Leeson and Caston, 2004). Hence, much effort has been made to search for fortifying lutein-rich egg yolk production via dietary supplements in laying hens. However, a very limited study was conducted to investigate the effect of dietary sources of lutein on the efficiency of incorporation of lutein into egg yolks in laying hens. To increase the efficiency of lutein bioavailability by means of dietary supplements in laying hens based upon the previous reports, it is thought that the dietary matrix is the most important factor that affects dietary lutein transfer into egg yolks. The lipid matrix of dietary components provided a better environment for lutein absorption, which stimulated the release of emulsifying bile acids by the gallbladder (Reboul et al., 2005). Another study also indicated that lutein from the emulsified lipid matrix was more readily absorbed into the blood than lutein from other sources, because of increasing intestinal absorption (Lakshminarayana et al., 2006). Moreover, several studies suggested that phospholipid micelles enhanced lutein bioavailability in rats and mice (Mamatha and Baskaran, 2011; Shanmugam et al., 2011).

Therefore, to maximize the deposition of lutein in egg yolks, it is crucial to investigate the effects of dietary matrix on the efficiency of lutein transfer into egg yolk. In this study, we selected a commercially available lutein and the emulsified crude extract of spinach as dietary supplements of lutein, which were adjusted to contain the same level of lutein concentration (40 mg lutein/kg of diet). In particular, a rich dietary source of lutein is spinach, which is known to contain about 6.3 mg of lutein per 100 g of wet basis (O’Neil et al., 2001).

The present study was conducted to investigate the effects of dietary lutein sources such as a commercial lutein and the emulsified crude extract of spinach on the transfer of lutein into egg yolks, egg qualities and hepatic antioxidant systems in laying hens.

Materials and Methods

Experimental Animals and Design

A total of thirty-six, 24-week old laying hens (Single Comb White Leghorn) were weighed and randomly allocated to three dietary groups; each group had 12 birds and was kept in wire cages in a room equipped with a temperature and on a light/dark cycle setting (light on 06:00–22:00). The laying hens were housed in commercial type cages (12 birds/cage, 871 cm²/bird) and the birds were fed a soy-corn-wheat basal diet formulated to meet all nutrient requirements for laying hens (Table 1). Immediately after the allocation of treatment, the three dietary groups were fed the basal diet supplemented with either nothing (CON), a commercial lutein dissolved into safflower oils (40 mg lutein/ kg diet), or the crude extract of spinach dissolved into safflower oils with lecithin (40 mg lutein/ kg of diet) for 5 weeks. The dietary level of lutein was chosen based upon a report by Egg Industry News (Shane, 2010), which indicated that a dietary xanthophyll content of at least 35 mg per kg of diet is required to obtain an acceptable Roche/DSM score of 7 to 8. All diets were prepared by mixing the basal diets with a corresponding pre-mixed additive according to an experimental design. The body weight was recorded on days 0 and 35 during the experiment to determine the effect of dietary lutein sources on body weight. On days 33 to 35, all eggs were collected for further analysis. The animal experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the university.

Preparation of Dietary Supplements with Lutein and the Crude Extract of Spinach

A commercial lutein 20% supplement was supplied in an

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>Basal diet</td>
<td></td>
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<tr>
<td>Ingredients (%)</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>41.25</td>
</tr>
<tr>
<td>Wheat ground</td>
<td>15.00</td>
</tr>
<tr>
<td>Soybean meal (44% CP)</td>
<td>25.00</td>
</tr>
<tr>
<td>DDGS*</td>
<td>5.00</td>
</tr>
<tr>
<td>Canola meal</td>
<td>2.00</td>
</tr>
<tr>
<td>Animal fat</td>
<td>0.50</td>
</tr>
<tr>
<td>Molasses</td>
<td>0.50</td>
</tr>
<tr>
<td>Granular ark shell</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.70</td>
</tr>
<tr>
<td>Limestone</td>
<td>8.70</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.20</td>
</tr>
<tr>
<td>Vitamin &amp; mineral premix †</td>
<td>0.15</td>
</tr>
<tr>
<td>Calculated chemical composition</td>
<td></td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>16.50</td>
</tr>
<tr>
<td>Ether extract (%)</td>
<td>2.90</td>
</tr>
<tr>
<td>Crude ash (%)</td>
<td>13.00</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>3.90</td>
</tr>
<tr>
<td>Available Phosphorous (%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Metabolic Energy (kcal/kg)</td>
<td>2,750</td>
</tr>
</tbody>
</table>

*DDGS: Distiller’s dried grains with soluble.
† Contained per kg: vit A, 7,000,000 IU; vit D3, 1,500,000 IU; vit E, 10,000 IU; vit K, 1,000 mg; thiamin, 1,000 mg; riboflavin, 3,000 mg; pyridoxine, 6,000 mg; vitamin B12, 18 mg; biotin, 40 mg; folic acid, 400 mg; Mg, 150 mg; Cu, 8,000 mg; Fe, 40,000 mg; Zn, 60,000 mg; Co, 100 mg; I, 1,000 mg; Co, 100 mg; Se, 250 mg; Mn, 90,000 mg.
oil form on a 79% safflower oil carrier that contained 1% dl-
α-tocopherol (FloraGLO® Lutein 20% SAF, DSM Nutri-
tional Products Inc., Basel, Switzerland). Lutein Oil Suspen-
sion 20% was added to the pre-mixture, which was finally
incorporated to the corn and soybean based diet at the
concentration of 40 mg of lutein/kg of diet (LUT group).
The crude extract process of air-dried powder of spinach was
prepared in the following manners. The whole leaves from
which roots were removed was cut into small pieces and then
air-dried for 48 hours at 60°C. After that, air-dry spinach
was crushed up and ground by a grinder to get a homo-
geneous powder and then kept in a dark place at room
temperature. To obtain crude extract, we immersed 1 part
of the air-dried spinach to 10 parts of ethanol (95%) for 48
hours. The Ethanolic layer containing the crude extract was
then concentrated using a rotary evaporator at 60°C to yield a
sticky greenish crude extract. The crude extract added to the
diet contained 6.15 mg of lutein per gram of the concentrated
greenish extract. In order to emulsify the crude extract of
spinach, the crude extract was mixed with the same volume
of safflower oil with 0.3% soy lecithin and vigorously agi-
tated for 2 hours (ECE group). Then, the emulsified crude
extract was added to the basal diet, which was adjusted for
the level of 40 mg lutein per kg of diet.

**Analysis of Egg Quality**

Twenty eggs from each group were randomly collected
during 3 days to test egg quality indices including albumen
height, Haugh units, yolk color, eggshell thickness and egg-
shell density. The egg quality was analyzed using a semi-
automated egg quality instrument (QCM+ Technical Ser-
tical Services and Supplies Co., York, UK). Each egg was carefully
weighed and broken onto a flat plate and the height of the
albumen was measured midway between the yolk and the
edge of albumen. Haugh unit (HU) was automatically ob-
tained by the conversion of the albumen height using the
Eggware software (TSS, York, UK). The index of egg yolk
color was determined using an electronic colorimeter (QCC
device, TSS) and was expressed in Roche scale points. Egg
shell density was calculated by TSS Eggware software.

**Analysis of Lutein by HPLC**

High-performance liquid chromatography (HPLC) was
used for analyzing the concentration of lutein in the crude
extract of spinach and egg yolks. The crude extract of
spinach was analyzed to estimate the exact amount of lutein
content. A total of twelve samples of egg yolks (n = 4) were
used for the analysis of the concentration of lutein using
HPLC. Each sample consisted of two homogenized egg
yolks from the same date. The homogenized yolks were
placed into 50 mL plastic tubes, wrapped with foil and stored
at −20°C until analyzed. For the preparation of the sample,
1 g of homogenized yolk was dispensed into a scintillation
vial. Nine milliliters of acetone was added and vials were
vortexed for 30 seconds. After setting for 1 hour at room
temperature, supernatant of the extract was filtered through a
0.45 μm syringe filter for HPLC analysis. The acetone was
evaporated under heat and the residue was dissolved into 1
ml of hexane (65): ethyl acetate (35). For the quantification
of lutein, an aliquot was injected into the HPLC system
(Shimadzu, LC-20AC pumps, a SPD-M20A diode array de-
tector) with LUNA C-18 column (5 μm, 4.6 mm × 250 mm).
The mobile phase consisted of methanol (90)/acetoni- trile +
triethylamine 9 μM (10) at a flow rate of 0.9 ml/minute. A
calibration curve was prepared using lutein standard (Sigma,
X6250, St. Louis, MO, USA).

**Tissue Harvesting**

After the 35-day feeding experiment, five birds per dietary
group (n = 5) were sacrificed by cervical dislocation. Imme-
 diately after sacrificing, the liver was harvested and the
weight of liver was expressed as a percentage of kg body
weight.

**Antioxidant Enzyme Activity and Lipid Peroxidation in the
Liver**

The homogenized liver tissues were centrifuged at 10,000
× g, and the resulting supernatant was centrifuged at 105,000
× g in a Centrikon T-2080 ultracentrifuge to harvest cytosol
and microsomes. The final pellet (microsomes) was sus-
pended in a phosphate buffer (pH 7.4) to obtain a protein
concentration of 20 mg/ml. The supernatant (cytosol) and a
suspended pellet (microsomes) were frozen in liquid nitrogen
and stored at −80°C until further assay. The activity of Cu-
Zn superoxide dismutase (SOD) was measured in the cytosol
fractions using xanthine and a xanthine oxidase for the
production of superoxide radical and subsequent measure-
ment of cytochrome c as a scavenger of the radicals
(Fridovich, 1974). The SOD activity was expressed as units/
mg of proteins, where 1 unit of activity was the amount of
enzyme required to inhibit the rate of reduction of cyto-
chrome c by 50%. Glutathione peroxidase (GPX) was de-
termined at 37°C in the cytosol fraction with cumene hydro-
peroxide as a substrate (Tappel, 1978). The GPX coupled
the reduction of cumene hydroperoxide to the oxidation of
NADPH by glutathione reductase, and concomitant oxidation
rate was measured with a spectrophotometer with the de-
crease in absorbance at 340 nm. One unit of GPX was ex-
pressed as the amount of GPX required to oxidize 1 μmol of
NADPH per minute. Glutathione S-transferase (GST) was
assayed with 1-chloro-2,4-dinitrobenzene (CDNB) as a sub-
strate by measuring the increase in absorbance at 340 nm
(Habig et al., 1974). One unit of activity was expressed as
the amount of GST catalyzing the conjugated CDNB per
minute. The lipid peroxidation in the microsome was mea-
sured by the level of 2-thiobarbituric acid (TBA) substances
with a spectrophotometer at 532 nm (Bidlack and Tappel,
1973). TBA substances are described in nanomoles of mal-
onaldehyde (MDA) per milligram of protein. Protein
concentration was assessed by the level of 2-thiobarbituric
acid (TBA) substances with a spectrophotometer at 532 nm
(Bidlack and Tappel, 1973). TBA substances are described in
nanomoles of malonaldehyde (MDA) per milligram of protein.

**Statistical Analysis**

The effects of dietary source of lutein on all data were
analyzed by Proc GLM (SAS Institute Inc., 1989). When the
dietary treatment was significant at P < 0.05, Tukey test was
applied to evaluate significant differences among dietary
groups. Data is presented as means ± SD.
Results

Body and the Relative Liver Weights

Body and the relative liver weights in laying hens fed a basal diet (CON) and diets supplemented with the commercial lutein (LUT) and the emulsified crude extract of spinach (ECE) are shown in Table 2. After 5-week feeding trial, there were no significant differences in body and the relative liver weights among laying hens fed a basal diet and the basal diet supplemented with various lutein sources.

The Quality of Eggs and Concentration of Egg Yolk Lutein

The quality of eggs and the concentration of yolk lutein in laying hens fed various dietary sources of lutein are presented in Table 3 and Figure 1, respectively. The laying hens fed diets containing a commercial lutein (LUT) and the emulsified crude extract of spinach (ECE) had a significantly (P<0.05) greater yolk color than those fed a basal diet (CON). However, the other egg quality indices such as whole egg weight, albumen height, Haugh units, yolk color, eggshell thickness and eggshell density were not affected by dietary group (Table 3). The concentration of egg yolk lutein was significantly (P<0.05) increased by dietary supplement with lutein or the emulsified crude extract of spinach compared with a basal diet (Fig. 1). The lutein content of egg yolk in the LUT group was found to contain approximately four times more lutein than that in the CON group. Although the concentration of yolk lutein was not statistically different

Table 2. Body weight and the relative live weight of laying hens fed basal diet (CON) and diets supplemented with the commercial lutein (LUT) and the emulsified crude extract of spinach (ECE)

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary group*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
</tr>
<tr>
<td>Initial BW (g), 24 wks</td>
<td>1243.6±128.8</td>
</tr>
<tr>
<td>Final BW (g), 29 wks</td>
<td>1436.3±142.6</td>
</tr>
<tr>
<td>Liver weight (g), g/100 g BW</td>
<td>2.81±0.85</td>
</tr>
</tbody>
</table>

*CON (the basal diet), LUT (a commercial lutein, 40 mg lutein/kg diet) and ECE (the emulsified crude extract of spinach, 40 mg lutein/kg diet).
Values of body weight are means±SD for 12 hens.
Values of relative liver weight are means±SD for 5 hens.

Table 3. The qualities of eggs in laying hens fed basal diet (CON) and diets supplemented with the commercial lutein (LUT) and the emulsified crude extract of spinach (ECE)

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary group*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
</tr>
<tr>
<td>Egg weight (g)</td>
<td>52.50±2.59</td>
</tr>
<tr>
<td>Egg color</td>
<td>69.95±2.69</td>
</tr>
<tr>
<td>Albumin weight (g)</td>
<td>7.99±1.06</td>
</tr>
<tr>
<td>Haugh unit</td>
<td>95.83±9.42</td>
</tr>
<tr>
<td>Yolk color</td>
<td>7.70±0.66b</td>
</tr>
<tr>
<td>Shell weight</td>
<td>7.42±8.56</td>
</tr>
<tr>
<td>Shell density</td>
<td>111.84±8.86</td>
</tr>
</tbody>
</table>

*CON (the basal diet), LUT (a commercial lutein, 40 mg lutein/kg diet), ECE (the emulsified crude extract of spinach, 40 mg lutein/kg diet).

\[ ^{a,b} \text{Values} (\text{mean±SD}, n=20) \text{with different superscripts are significantly different among dietary groups (} P<0.05).\]

Fig. 1. The content of lutein in eggs of laying hens fed basal diet (CON) and diets supplemented with the commercial lutein (LUT) and the emulsified crude extract of spinach (ECE). Means (Mean±SD, n=4) with different superscript differ among dietary groups (P<0.05).
between the two dietary sources of lutein, the LUT group showed a higher value of yolk lutein and a lower standard deviation of average yolk lutein, suggesting that dietary supplements with a commercial lutein could be more effective and evenly transferring lutein into yolk than the crude extract of spinach in laying hens.

**Antioxidant Enzyme Activity and Lipid Peroxidation in the Liver**

To determine whether a dietary source of lutein affects antioxidant defense system in the liver in laying hens, we examined the specific activities of SOD, GPX and GST and the level of lipid peroxidation (Fig. 2). The specific activity of hepatic SOD in the LUT group was significantly ($P<0.05$) higher than that in the CON and ECE groups, whereas GPX and GST activities were not affected by a dietary source of lutein. The lipid peroxidation as indicated by MDA level in the liver was also unaffected by various sources of dietary lutein in laying hens (Fig. 2, D).

**Discussion**

The primary aim of the present study was to compare the transfer of lutein into egg yolks and the hepatic antioxidant system in laying hens fed diets supplemented with a commercial lutein and the emulsified crude extract of spinach. In this study, the concentration of lutein in egg yolks from the laying hens fed diets from either sources of lutein were significantly higher than those from the laying hens fed a basal diet. In addition, the laying hens fed diet containing the emulsified crude extract of spinach had egg yolk with a similar lutein concentration compared with those fed diet supplemented with lutein. This result was expected, as a dietary supplement with xanthophylls is considered to be easily transferred to egg yolks in laying hens. It is well demonstrated that dietary carotenoids including lutein are incorporated to egg yolks and subsequently contribute to egg yolk color in laying hens (Lee and Summers, 1997).

In general, lutein supplements fed to human and rodents are highly affected by the dietary matrix (Toyoda et al., 2002), indicating that dietary components and types of supplements affect lutein transfer into the eggs in laying hens. However, little is known about lutein bioavailability by which dietary source of lutein modulates the incorporation of lutein into egg yolks in laying hens, although it was known that the dietary matrix affected lutein bioavailability in mice and rats. There are a series of steps associated with the bioavailability of lutein from dietary supplements. These steps are involved in the release of lutein from the dietary matrix, the transfer of lutein to micelles, the absorption of lutein by intestinal membrane, and the transport of lutein to the blood and egg yolks (Zaripheh and Erdman, 2002). In these steps, it is thought that the transfer of lutein into egg yolks from laying hens fed a diet containing the crude extract
of spinach is enhanced by the supplementation of oil with phospholipid, which activates the emulsification of the diet matrix and enhances intestinal absorption.

According to several reports with human and rodent (Mamatha and Baskaran, 2011; Zaripheh and Erdman, 2002), the absorption of lutein through the small intestine was maximized in the presence of dietary fat, which activated the release of emulsifying bile acids in the gut (Zaripheh and Erdman, 2002). Castenmiller et al. (1999) reported that dietary carotenoids dissolved in oil were more readily absorbed when they were added to diets with vegetables in human. In addition, it was reported that phospholipid, an effective biosurfactant, enhanced the absorption of lutein by altering the permeability of the cell membrane (Hauser, 2000; Marsid-daiah and Baskaran, 2009). This increase in lutein bioavailability is in agreement with the observations of Shammugan et al. (2011) who reported that dietary supplements with lutein containing phosphatidylcholine as oil phase significantly increased the bioavailability of lutein in rabbits. In our study, thus, one possible explanation for increased egg yolk lutein in the ECE group was the matrix of crude extract of spinach, which contained a mixture of safflower oil with soy lecithin. Lutein from the emulsified crude extract of spinach would be absorbed comparable to a commercial lutein in laying hens under the circumstances of this study. In particular, lutein was more efficiently incorporated into the polar exterior of micelle, since it has a higher polarity than any other carotenoids (Deming and Erdman, 1999). In fact, the bioavailability of lutein from spinach ranged from 45 to 52%, whereas that of β-carotene from spinach ranged from 5.1 to 9.5%, which indicated that the bioavailability of lutein was much greater than other carotenoids and less affected by the dietary sources (Castenmiller et al., 1999). From the previous studies with human and rodent, lutein is known to be more readily bioavailable from dietary sources than any other carotenoids (Zaripheh and Erdman, 2002). Therefore, in this study, those reasons might explain why laying hens fed a diet containing emulsified crude extract of spinach showed a similar extent of yolk lutein content compared with those fed a diet containing a commercial lutein.

However, it should not be ignored that the LUT group showed numerically higher and much lower variability of yolk lutein content than the ECE group, although there was no statistical difference in egg yolk content between the two groups. This may imply that dietary supplements with lutein could be more consistently transferred into yolk than that with the crude extract of spinach in laying hens. In other words, dietary supplements with a commercial lutein to laying hens could produce more consistency quality of lutein-enriched eggs.

To evaluate the effects of dietary lutein sources such as a commercial lutein or the emulsified crude extract of spinach containing lutein on antioxidant defense system, we examined antioxidant enzymes (SOD, GPX and GST) and lipid peroxidation (MDA) in the liver of laying hens. The laying hens fed a diet supplemented with lutein resulted in a significant increase in SOD activity. Hence, it is speculated that increased SOD activity in response to feeding a commercial lutein may act as antioxidant scavenger against oxidative damage in the liver of laying hens. However, there was no significant effect of emulsified crude extract of spinach on the hepatic antioxidant defense system in laying hens. It seemed that laying hens fed a diet supplemented with lutein were more effective in hepatic antioxidant system than those fed a diet supplemented with the crude extract of spinach in this study. This could be explained by the fact that a commercial lutein could be more consistently absorbed than lutein from the emulsified crude extract of spinach in laying hens.

The antioxidant systems, both the non-enzymatic free radical scavengers such as carotenoids and the endogenous antioxidant enzymes including SOD, GPX and GST have responsibility for the protection of cellular membrane against excess oxidative radicals (Kristal et al., 1997). Some dietary antioxidants actively modulate endogenous antioxidant enzymes to remove excessive reactive oxygen species induced by oxidative stress (Chen et al., 1987). Carotenoids such as lutein are an effective chain-breaking antioxidant because of their ability to quench singlet oxygen and scavenge peroxo radicals (Wang et al., 2006). However, there is a lack of information on the effects of dietary lutein on endogenous antioxidant enzymes in laying hens, although numerous in vitro studies have demonstrated that lutein has shown beneficial effects on antioxidant defense system against free radicals (Bhattacharyya et al., 2010; Hayes et al., 2009; Li et al., 2007). In agreement with our study, a dietary feeding of lutein in vegetable oils to rats increased lutein absorption, which in turn increased antioxidant enzymes, suggesting that lutein bioavailability might be a crucial factor that affects the antioxidant system in animals (Lakshiminarayana et al., 2009). Sindhu et al. (2010b) also reported that the oral administration of lutein (50, 100 and 200 mg lutein/kg of BW) in mice significantly enhanced the activity of SOD, GST and GPX in the liver. Therefore, it seems that a dietary supplementation of lutein may have beneficial effects on oxidative stability through modulating endogenous antioxidant enzymes in laying hens. Interestingly, it was reported that dietary lutein (50 mg/kg diet) reduced hepatic lipid peroxidation in turkeys injected with lipopolysaccharides (LPS) without affecting lipid peroxidation in those with no LPS injection, suggesting that dietary lutein (50 mg lutein/kg of diet) has an antioxidant ability for oxidative damage under stressful conditions in poultry (Shammugasundaram and Selvaraj, 2011). Similarly, a study with rats reported that antioxidant enzymes including SOD and GPX were found to be enhanced in lutein-supplemented rats compared with control group when hepatic damage was induced by alcohol and CCL4 (Sindhu et al., 2010a). In view of oxidative stress, another possible reason for increased SOD activity in the LUT group was that the dietary matrix of safflower oil containing high level of unsaturated fatty acids, which was used for dissolving a commercial lutein might produce free radicals to activate SOD for the protection from oxidative...
reaction.

By contrast, several studies reported that dietary lutein did not directly affect glutathione status in the liver of rats (Jetwell and O’Brien, 1999) and erythrocyte antioxidant enzyme activity of SOD and GPX in human (Hininger et al., 2001). This observation of unaffected glutathione status might explain that GPX activity was not affected by the supplementation of a commercial lutein in our study, since glutathione status is directly associated with GPX activity. More sophisticated study is still needed to elucidate the effect of a dietary source of lutein on the antioxidant defense system in laying hens due to complicated factors such as lutein bioavailability, and internal and external conditions.

Taken together, laying hens fed a diet containing lutein from either source significantly increased the lutein concentration of egg yolks and color of yolk compared with those fed a basal diet. Laying hens fed a diet supplemented with lutein showed a significant increase in hepatic SOD compared with those fed a basal diet. Conclusively, the dietary supplementation of the commercial lutein showed more consistency in the quality of lutein-enriched eggs and improved hepatic SOD activity compared with that of the emulsified crude extract of spinach, although both dietary lutein sources can be potential candidates to produce egg-enriched lutein in laying hens.

Acknowledgments

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