Quantification of lutein in egg following feeding hens with a lutein supplement and quantification of lutein in human plasma after consumption of lutein enriched eggs

Anreicherung von Lutein im Ei durch Fütterung der Hennen mit einem Luteinzusatz und Plasma-Luteinspiegel beim Menschen nach dem Verzehr der angereicherten Eier

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Introduction

Lutein and zeaxanthin belong to the large class of plant pigments referred to as carotenoids. Their polarity is greater than many other carotenoids due to the presence of hydroxyl groups on the cyclic ring structure. Unlike provitamin A carotenoids (α- and β-carotene and cryptoxanthin), they can not be converted to vitamin A (MARES-PERLMAN et al., 2002). Lutein and zeaxanthin are present in a wide variety of fruits and vegetables and they cause the yellow colour in some plants, such as in corn. Their concentration is particularly high in leafy green vegetables such as spinach, collards and kale. They are also present in some animal products such as egg yolks due to plant products eaten by birds (MANGELS et al., 1993). Currently, in humans it is speculated that consuming higher levels of these carotenoids leads to increased levels in body tissues, particularly in eyes, and that this may confer health benefits by lowering the risk of chronic diseases (MARES-PERLMAN et al., 2002).

Lutein and zeaxanthin are major serum carotenoids in humans, along with α- and β-carotene and lycopene (PARKER, 1989). Dietary intake of foods which are rich in these carotenoids has been shown to influence serum concentrations in a positive manner (HAMMOND et al., 1997; BONE et al., 2000). However, individual variation in serum response to increased intake has been observed and may be due to factors such as varying rates of absorption and tissue uptake.

Metabolites of lutein and zeaxanthin have been identified in human blood and tissues (HAMMOND et al., 1997). It is reported that lutein ester provides positive effects in human tissues (WINGERATH et al., 1998). However, only the free form (non esterified) appears in serum after the intake of xanthophyll esters under physiologic conditions (BREITHAUPF et al., 2003). BOWEN et al. (2002) reported higher bioavailability from lutein ester formulation compared with the free form although this difference was not significant. On the other hand CHUNG et al. (2004) speculated that humans have a very efficient hydrolysis system for xanthophyll esters and that ester hydrolysis is not the limiting step for lutein ester absorption. They confirmed that lutein bioavailability from lutein, lutein ester supplements, and spinach did not differ.

A prevalent isomer in human retina, mesozeaxanthin, may be metabolized from dietary lutein (BONE et al., 2000). The biological plausibility that lutein and zeaxanthin protect against the development of cataract and macular degeneration is supported by the fact that they have chemical properties that may retard pathogenic mechanisms which are thought to promote these degenerative conditions. Oxidative stress is high in the eye due to the intense light exposure and the high rate of oxidative metabolism in the retina (MARES-PERLMAN et al., 2002). YOUNG and LOWE (2001) reviewed the antioxidant properties of lutein and zeaxanthin. However, KRNISKY (2002) in a separate investigation cited the lack of direct evidence for antioxidant protection of these carotenoids in vivo. Another potential animal model which was used to study the protective effects of lutein and zeaxanthin on the retina was the quail. Quail retina, like the primate macula, is dominated by cone photoreceptors and concentrates lutein and zeaxanthin. Preliminary studies indicated an inverse correlation between the level of zeaxanthin in quail retina and light induced retinal cell death (DOBEY et al., 1997). Lower risk for macular degeneration has been associated with the consumption of food sources of these carotenoids, with overall levels of lutein and zeaxanthin in the diet or with higher levels of these carotenoids in the blood or retina (MARES-PERLMAN et al., 2002). Lutein and zeaxanthin may play a role in the health of other body tissues, as well. Although these relationships are largely unexplored, there is the possibility that lutein, together with other carotenoids, may protect against cancer, cardiovascular disease and other conditions which may involve the immune system. The effect of lutein on immune system might reside in its antioxidative properties. KOUTZOS et al. (2003) reported that lutein is present in immune cells of chickens and that carotenoid deposition in immune tissues of growing chicks is influenced by maternal diet composition, indicating that dietary lutein could be efficiently transferred from breeder hens to their offspring. Lutein may enhance monocyte function by increasing the number of surface molecules expressed by monocytes (HUGHES et al., 2000). Lutein and
lutein’s safety as a nutrient (ALVES-RODRIGUES and HAO, 1999). HANDELMAN et al. (1991) showed that egg yolk is a highly bio-available source of lutein and zeaxanthin. The benefit of introducing these carotenoids into the human diet with egg yolk is counterbalanced by potential LDL-cholesterol elevation from the added dietary cholesterol.

Egg yolk is a matrix composed of digestible lipids, cholesterol, triacylglycerol, and phospholipid. Lutein and zeaxanthin are dispersed in this matrix along with other fat-soluble micronutrients such as vitamins A, D and E. Lutein and zeaxanthin in egg yolk might be highly bio-available because of their association with the lipid matrix of the egg yolk (SCHAEFFER et al., 1988). LAI et al. (1996) found that colour of egg yolk from hens fed the same levels of free and esterified red pepper carotenoids are not significantly different, indicating a nearly identical absorption tendency of carotenoids, whether free or originally esterified. Carotenoids have been used for many years in the poultry industry as a means of pigmenting eggs (LEESON and SUMMERS, 1997). LANDRUM and BONE (2001) suggested that the intake of lutein and zeaxanthin by humans is less than 1 mg/d, which is much lower than the preventive levels being tested for, currently (GRANDO et al., 2003). Eggs normally contain 0.3 to 0.5 mg of total xanthophylls, with just over half present as lutein (STEINBERG et al., 2000). There is limited information available on the efficiency of transport of various xanthophylls into the egg and the factors that influence such deposition. Efficient transfer of lutein into eggs is affected by dietary composition (BEDECARRATS and LEESON, 2006). However, recent reports indicated that egg yolk is a highly bioavailable source of lutein, increasing serum lutein concentration 110-350 nmol/L for each milligram of lutein ingested (CHUNG et al., 2004). There is little data on the toxicity of lutein. Lutein and its ester form administered orally at doses of 4, 40, and 400 mg/kg body weight for 4 weeks for short-term toxicity study and 13 weeks for a subchronic toxicity study did not produce any mortality, change in body weight, food consumption pattern, organ weight, and did not show other adverse side effects. Administration of free lutein and its ester form did not alter the hepatic and renal function, and did not produce any change in the haematological parameters and in lipid profile in Wistar rats. Histopathological analysis of the organs supported the non-toxicity of lutein and its ester form (HARKUMAR et al., 2008).

Materials and Methods
In order to study the effect of dietary lutein on lutein and zeaxanthin concentration in the egg this research was conducted in a completely randomized design, consisting of four treatments each in four replicates. The total numbers of laying hens used in this experiment were eighty commercial hybrids of Single Comb White Leghorn (5 hens per cage). The experiment was performed in accordance with ethical commissions for animal welfare of Iran Agricultural Ministry. A Corn-soybean meal based diet was formulated for layers at 32-37 weeks of age (from Aug 2008 to Sep 2008) and all diets were prepared in an all-mash form (Table 1). Before starting the experiment birds were fed the control diet (Table 1) and at the commencement of the trial four levels of lutein (0, 250, 500, 750 mg/kg; LEESON and CASTON, 2004; BEDECARRATS and LEESON, 2006) were given as supplied by DSM Company (Istanbul, Turkey; FloraGlo 10% lutein, commonly used in the human food). The concentrations of lutein and zeaxanthin in the

### Table 1. Composition and nutrient content of the experimental ration (%)

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Corn</td>
<td>49.42</td>
</tr>
<tr>
<td>Soy bean meal</td>
<td>9.78</td>
</tr>
<tr>
<td>Wheat</td>
<td>25</td>
</tr>
<tr>
<td>Fish meal</td>
<td>5</td>
</tr>
<tr>
<td>Salt</td>
<td>0.22</td>
</tr>
<tr>
<td>Di calcium phosphate</td>
<td>0.57</td>
</tr>
<tr>
<td>Min + vit. premix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>Di-methionine</td>
<td>0.06</td>
</tr>
<tr>
<td>Oyster shell</td>
<td>8.75</td>
</tr>
<tr>
<td>Calculated analysis</td>
<td></td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>2800</td>
</tr>
<tr>
<td>Crude protein</td>
<td>14.48</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.65</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.29</td>
</tr>
<tr>
<td>Methionine + cystin</td>
<td>0.56</td>
</tr>
<tr>
<td>Ca</td>
<td>3.14</td>
</tr>
<tr>
<td>P (nonphytate)</td>
<td>0.24</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>1.53</td>
</tr>
<tr>
<td>Na</td>
<td>0.14</td>
</tr>
<tr>
<td>Lutein&lt;sup&gt;2&lt;/sup&gt; (mg/kg)</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Zeaxanthin&lt;sup&gt;2&lt;/sup&gt; (mg/kg)</td>
<td>5 ± 1</td>
</tr>
</tbody>
</table>

<sup>1</sup> Supplied (per kilogram of premix): vitamin A, 6,000,000 IU; cholecalciferol, 1,500,000 IU; vitamin E, 15,000 mg; riboflavin, 3,000 mg; pantothenic acid, 7,000 mg; niacin acid, 25,000 mg; folic acid, 500 mg; and vitamin B12, 15,000 IU; thiamine, 3,000 mg; pyridoxine, 500 mg; vitamin B6, 3,000 mg; biotin, 100 mg; inositol, 100 mg; Fe, 30,000 mg; Mn, 120,000 mg; Zn, 80,000 mg; Co, 600 mg.

<sup>2</sup> Determined concentration.
feed were analyzed in the diet by High Performance Liquid Chromatography (HPLC) according to the methods of WEBER (1988) and LEESON et al. (2007) (Table 1). Daily records were made of egg production. At the end of every week (after a week of adaptation) all eggs were collected for assessment of yolk colour score using the DSM Yolk Colour Fan (previously called Roche Yolk Colour Fan) of 1 to 15. At the end of the trial two eggs were collected from each replicate to assay the lutein and zeaxanthin content by HPLC as previously described by LEESON and CASTON (2004). A calibration curve was prepared using lutein and zeaxanthin standards (LEESON and CASTON, 2004).

The effect of dietary supplementation with lutein enriched eggs was examined in 4 men per treatment with a mean age of 60 years (range: 56–64 years old). Eggs were produced by hens fed on diets with different levels of lutein as described above (0, 250, 500, 750 mg/kg). The trial was performed in accordance with Iran Committee of Bioethics, National Committee for Ethics in Science and Technology. The subjects were non-smokers and were not using any medications. Persons with a history of active small bowel disease or resection, atrophic gastritis, insulin requiring diabetes, alcoholism, pancreatic disease, or bleeding disorders were excluded from the study. The men received one egg in their daily diet for 4 weeks and during the experiment they were fed with the same diet. Blood samples were collected 1 week before the treatment (week -1). As the qualitative and quantitative profiles of carotenoids, their metabolites, vitamins A and E in the sera of each subject at the two time points were nearly identical, the data from week -1 were used as baseline measurements (KHACHIK et al., 2006). In addition, blood samples were collected at the end of this period, and morning blood samples were collected after a 8 hours fasting. Plasma lutein was analysed by using a procedure adapted from the method of HANDELMAN et al. (1999).

**Results and Discussion**

Data for yolk colour score, as well as lutein and zeaxanthin of egg yolk are presented in Figure 1 and Table 2, respectively. The results of dietary egg yolk enrichment with different levels of lutein on men plasma lutein, who received one egg per day, are presented in Figure 2.

After one week of feeding with lutein supplementation, the lutein and zeaxanthin of the egg yolk increased significantly (p < 0.01) (Table 2). The most noticeable enrichment occurred for 250 mg/kg of lutein and the highest level of egg yolk lutein and zeaxanthin was reached with 750 mg/kg lutein. No significant differences were observed in yolk lutein content between supplementation of 500 and 750 mg/kg lutein to the diet.

After 7 days of supplementation, egg yolk colour increased from 5.5 to 13.7 on the DSC scale. The yolk colour in groups fed with 250 mg/kg lutein during the trial increased, but egg yolk colour was not affected widely by dietary lutein supplements above 500 mg/kg (p < 0.05) (Figure 1). The occurrence of lutein in egg yolk is in agreement with observations by LEESON and CASTON (2004) who reported that adding lutein to the layers’ diet resulted in a significant (p < 0.01) increase in Roche color score of yolk within 7 d of supplementation. TOYODA et al. (2002) showed in their research on quail that the average concentration of lutein and zeaxanthin in female serum and liver exceeded those in males by 3 to 10 fold, but the mean concentration of lutein and zeaxanthin in female retina and fat were at most two and four times higher than those in males, respectively. This difference is explained by the deposition of lutein and zeaxanthin in the eggs due to the fact that females laid continuously. BREITHAUPT et al. (2003) indicated that after feeding commercial laying hens with free lutein supplemented for one week, the carotenoid profile of the plasma changed.

Lutein and zeaxanthin, natural xanthophylls not synthesized by the human body, have been investigated for their use in promoting visual health (ZHANG and SWEET, 2008). Plasma lutein was high after consumption of eggs supplemented with 500 and 750 mg/kg lutein (p < 0.05). Plasma lutein level of men in the present study is in line with the work of CHUNG et al. (2004) in which a greater serum lutein response was observed after egg consumption compared with supplements and spinach. ZHANG and SWEET (2008) reported that increased dietary intake of lutein and zeaxanthin resulted in increased plasma levels, which were positively and significantly associated with macular pigment optical density. Limited data have suggested that supplementation may also improve visual function. The optimal dose of lutein and zeaxanthin for the prevention or treatment of age-related macular degeneration (AMD) has not

**Statistical Analysis**

The data were analysed by completely randomized design method with four treatments each applied to the same number of units and four replications per treatment. The SAS program (SAS INSTITUTE, 1996, SAS Institute Inc., Cary, NC, USA) was used for analysing data. The means of variables were compared using Duncan’s multiple range test (DUNCAN, 1955). The test was performed at 95 and 99% Confidence Intervals (CIs), and p < 0.05 and p < 0.01 were taken as significant differences, respectively. The results presented are means ± SD. A paired t-test was performed to study the increase in plasma lutein concentration from base line in humans.

<table>
<thead>
<tr>
<th>Dietary lutein (mg/kg)</th>
<th>Lutein</th>
<th>Zeaxanthin</th>
<th>Lutein + Zeaxanthin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.12± 0.03</td>
<td>0.14± 0.02</td>
<td>0.26± 0.07</td>
</tr>
<tr>
<td>250</td>
<td>1.35± 0.06</td>
<td>0.12± 0.08</td>
<td>1.47± 0.09</td>
</tr>
<tr>
<td>500</td>
<td>1.41± 0.05</td>
<td>0.11± 0.05</td>
<td>1.52± 0.03</td>
</tr>
<tr>
<td>750</td>
<td>1.43± 0.08</td>
<td>0.11± 0.06</td>
<td>1.54± 0.06</td>
</tr>
</tbody>
</table>

*a,b,c* Mean values in the same column with different superscript letters were significantly different.

**p < 0.01**.
yet been defined. ATA et al. (2009) in a trial with humans confirmed that egg is a good vehicle for dietary carotenoid absorption and that increased dietary lutein correlates with macular pigment density and not with deposition of carotenoids in skin. In egg yolk, lutein is located in the digestible lipid matrix, which is composed of cholesterol, triacylglycerol and phospholipids. The cholesterol content of egg yolk may enhance the bioavailability of lutein (COTTERIL et al., 1977). Dietary intake of food in these carotenoids has been shown to influence serum concentration in a positive manner (HAMMOND et al., 1997). TANG et al. (1995) found that long-term canthaxanthin supplementation in ferrets reduced lutein-zeaxanthin in fat, whereas data from GROLIER et al. (1997) indicated that canthaxanthin interfered with absorption or processing of β-carotene. When MICOZZI et al. (1992) fed a broccoli supplement providing 6 mg lutein per day, plasma lutein values returned to baseline 4 weeks after the dietary broccoli supplementation was stopped. Reported concentration of lutein and zeaxanthin are often highest in macular tissue, the proportion of total carotenoids in tissue comprised of lutein and zeaxanthin varies across individuals may reflect both diet and genetic factors. This indicates that diet will influence lutein and zeaxanthin absorption and that increased dietary lutein correlates with macular pigment density with feeding of foods (HAMMOND et al., 1997). TANG et al. (1995) found that long-term canthaxanthin supplementation in ferrets reduced lutein-zeaxanthin in fat, whereas data from GROLIER et al. (1997) indicated that canthaxanthin interfered with absorption or processing of β-carotene. When MICOZZI et al. (1992) fed a broccoli supplement providing 6 mg lutein per day, plasma lutein values returned to baseline 4 weeks after the dietary broccoli supplementation was stopped. Reported concentration of lutein and zeaxanthin are often highest in macular tissue, the proportion of total carotenoids in tissue comprised of lutein and zeaxanthin varies as well (CHUNG et al., 2004). The level of variation across individuals may reflect both diet and genetic factors. This indicates that diet will influence lutein and zeaxanthin concentration, which is supported by several investigations who have reported increases in macular pigment density with feeding of foods (HAMMOND et al., 1997) or supplements (LANDRUM et al., 1997) rich in these carotenoids.

The current study showed that egg yolk can provide a highly bio-available source of lutein and zeaxanthin and by supplementing the diet with lutein it is possible to elevate it in egg yolk. These findings indicate that in human adults, consuming one egg per day for four weeks, the concentration of plasma lutein content increases significantly.

**Summary**

Two experiments were designed in order to study the effect of four dietary levels of lutein on egg yolk lutein, and zeaxanthin and lutein concentrations in the plasma of male participants after consuming these enriched eggs. In experiment one, 80 single comb White Leghorn commercial hybrids were randomly distributed in four groups with four replicates. Each replicate consisted of 5 hens per cage. Four levels of lutein (0, 250, 500, 750 mg/kg) were fed as treatments in a completely randomized statistical design. In experiment two, four men (per treatment) with the average age of 60 years, were fed one enriched egg per day for 4 weeks. The lutein was transferred into the yolk (p < 0.01). Seven days after supplementation, the DSM scale of the yolk's colour significantly increased from 5.5 to 13.7. The plasma content of lutein was significantly increased in human adults who consumed one egg per day of treatments 500 and 750 mg/kg (p < 0.05).

**Key words**

Human, diet, egg yolk, laying hen, lutein, plasma

**Zusammenfassung**

Anreicherung von Lutein im Ei durch Fütterung der Hennen mit einem Luteinzuatz und Plasma-Lutein­spiegel beim Menschen nach dem Verzehr der angereicherten Eier


**Stichworte**

Mensch, Legehenne, Nahrung, Dotter, Plasma, Lutein
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