Availability of lutein and lycopene from enriched egg yolk powder in mice
Annamária Kerti, Zsuzsanna Kiss, Csaba Szabó and László Bárdos
Department of Animal Physiology and Health, Faculty of Agriculture, Szent István University
2103. Páter K. s. 1. Gödöllő, Hungary
kerti.annamaria@mkk.szie.hu

Introduction
Human and animal investigations have demonstrated that carotenoids possess a range of important and well documented biological activities. Carotenoids are powerful antioxidants, potential anti-cancer and anti-aging compounds; they take part in the immunological processes (RAO and RAO, 2007).
One of the most investigated roles of carotenoids is the preventive and curative effect of two oxycarotenoids (lutein and zeaxanthin) for protection of the macula from degeneration (SEDDON et al., 1994). Age related macular degeneration (AMD) affects older adults that cause central vision loss because of damage to the macula lutea (“yellow spot”) of retina. The yellow appearance, and the name macula lutea, is due to the dense concentration of lutein and zeaxanthin. Other carotenoids are not found here (KHACHIK et al., 2002). These yellow dies protect the retina from harmful energy of light. Without this protection oxidative stress within the retina is leading to a loss of central vision and develops the AMD. The mechanism by which lutein is effective in preventing eye disease is not known, but may involve its role as an antioxidant through its ability to quench free radicals and singlet oxygen and by absorbing blue light.
Age-related macular degeneration is a disease associated with aging that gradually destroys the macula. AMD is an untreatable disease and occurs in “dry” and “wet” forms. It is the leading cause of irreversible blindness among the elderly (in people over age 50) across the world. The prevalence of AMD and the associated social and economic consequences of blindness from AMD are increasing as the number of older people in our population continues to increase. However, despite the significance of this disease, there are no available means to prevent it.
There are a number of risk factors which have been identified playing role in developing AMD, such as age, gender, genetics, smoking, sunlight and nutrition. It is possible to control the environmental factors seem to be linked to AMD. There is no cure for macular degeneration, so utilising all means of prevention, including nutrition intervention is extremely important. Along with their lutein and zeaxanthin content, eggs are a natural source of at least 10 different vitamins and minerals, making them a nutrient-rich food. This means they not only may assist with reducing the risk of macular degeneration, they also make a positive contribution to an individual’s diet.
The ability to increase the amount of macular pigment by dietary supplementation with lutein has been demonstrated. Consuming a well-balanced diet with plenty of fresh fruits and vegetables containing oxycarotenoids may help to delay the progress of AMD (TRUMBO and ELLWOOD, 2006). It has been suggested that eating green leafy vegetables (such as spinach, kale, and broccoli), which are rich in lutein and zeaxanthin, may decrease the risk for AMD (KRINSKY et al., 2003, SOMMERBURG et al., 1998).
Beside the green vegetables and supplements the egg yolk contains lutein and zeaxanthin in considerable amount. The consumption of one egg daily elevates the levels of antioxidants lutein and zeaxanthin compared with no egg consumption, therefore can reduce the risk of developing macular degeneration. However egg yolks contain considerably less lutein than some plant sources, but recent reports indicated that the lutein bioavailability from egg is higher than that from vegetable sources (CHUNG et al., 2004).
Lycopene is an acyclic carotenoid, one of more powerful antioxidants, which is found in several fruits and vegetables (red grapefruit, watermelon, guava, rose hips and tomato), and microorganisms that help neutralize free radicals that are believed to cause heart disease, cancer in special disease of prostate. Lycopene has a lot of health benefits as antioxidant, cell cycle modulator; it has hypocholesterolemic and anticarcinogenic activity especially in prostate cancer. This carotenoid is not a common constituent of poultry fodder. Our previously published results indicate that dietary lycopene can be accumulated into yolk and resulting its desired colour. Taking into account the health benefits of the lycopene enriched egg it may be a candidate for functional food (GREGOSITS et al., 2009).

On the base of these finding an investigation was carried out to determine whether the egg yolk powder as an endurable egg product can be offered as a permanent source for lutein and lycopene.

Material and methods

Feed additives

The experimental feed was supplemented with lutein and lycopene: either with egg yolk powder containing these chemicals or with gelatine coated formulations (Lutein 5% CWS/S-TG; Redivivo™ (lycopene) 5% CWS/S both DSM, Nutritional Products Ltd. Basel, Switzerland) in parallel.

Preparation of fortified egg yolk powder

Commercial eggs were broken and an aliquot volume of yolk was mixed either with lutein or lycopene or with both of them to final concentration 1% (v/w). These mixtures were pulverized with Mini Spray Drier B-191 (BÜCHI Labortechnik GmbH, Germany).

Animals and experimental set-up

BALB/c inbreeds (Charles River Ltd, Isaszeg, Hungary) laboratory mice were used in the experiment. Eight groups were arranged with 10-10 animals (average weight: 25.4±5.7 g) in each. Animals were fed ad libitum with a basic and/or supplemented feed. Basal diet used was laboratory mice feed. After grinding, this feed was mixed with either antioxidant containing egg yolk powder or with microcapsulated preparations. Two control diets were formulated. One (A) was prepared without any supplementation and the other (B) with commercial (not fortified) egg yolk powder.

The animals were fed ad libitum for 10 days. Table 1 contains the experimental and feeding set-up. Five mice from each group were picked out and lege artis sacrificed on the 5th and 10th days after the beginning of dietary supplementations. Blood sera and livers were obtained for chemical analyses.

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Lutein % in the feed (w/w)</th>
<th>Lycopene</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>C-0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>C-EYP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Lu-EYP</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Lu-MCF</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Ly-EYP</td>
<td>0.035</td>
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<tr>
<td>F</td>
<td>Ly-MCF</td>
<td>0.035</td>
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<tr>
<td>G</td>
<td>LuLy-EYP</td>
<td>0.035 0.051</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>LuLy-MCF</td>
<td>0.035 0.051</td>
<td></td>
</tr>
</tbody>
</table>

Lu=lutein, Ly=lycopene, EYP=yolk powder, MCF=microcapsulated formulation
**Chemical analysis**

Lutein and lycopene concentrations of egg yolk powders, sera and liver samples were analyzed by HPLC techniques adapted and modified in our laboratory (KERTI and BÁRDOS, 2006). The procedure briefly: the samples were extracted with extraction mixture (hexane: acetone: ethanol: toluol 10:7:6:7) and saponified in alcoholic potassium hydroxide (56°C for 30 min). After the extract got cold at room temperature in dark for one hour it was mixed with hexane again. After the solution was placed in darkness for one hour again 400 µl of supernatant was evaporated in N₂ stream. The residuum was dissolved in 100 µl of ethanol/dioxane (1:1) and in 150 µl acetonitrile. The clear extract (20 µl) was injected onto C18 Rocket Platinum column (100A 3µ 53 mm x 7 mm)(Alltech, USA) that was connected to the HPLC system (PU-980 pump, UV-2077 4λ four channel detector (Jasco, Japan)). The mobile phase was prepared of mixtures of acetonitrile: tetrahydrofuran: methanol: ammonium acetate 1% (684:220:68:28). The flow rate was 1 ml/min and the chromatograms were monitored at 450 nm in case of lutein and 505 nm in case of lycopene. Identification of abilities based on standards and the concentrations were calculated by Chrompass software (Jasco, Japan).

**Statistical analysis**

Data were analysed by one-way ANOVA with Turkey’s multiple comparison tests that were performed using GraphPad Prism version 5.0 for Windows.

**Results and discussion**

There was no difference in serum and liver lutein concentrations between the control (A) group and those who consumed the not fortified egg yolk powder containing feed (B). Namely the low lutein concentration of commercial egg yolk powder was not enough for the elevation of the blood lutein level and liver storage. The situation was the same in those groups which received lycopene supplementation regarding lutein concentrations. The groups that received lutein supplementations (by fortified egg yolk powder and by microcapsulated formulation) showed significantly (p<0.001) higher lutein concentrations in the blood already at the 5th days. The elevation in blood lutein concentration was more appreciable in both groups at the 10th days (Figure 1). But there is a difference between the supplemented groups: the carotenoid supplementation in egg yolk powder resulted higher values than the application of the pure DSM product. This was probably because of favourable bioavailability of egg yolk. The joint supplementation of carotenoids does not influence the absorption of lutein; the application of the same doses was little bit more advantageous.

![Figure 1](image-url)

**Figure 1**
Lutein and lycopene concentrations of sera on 5th and 10th days (Mean±SEM)(µg/L)
Liver storage of lutein at the 5th days was higher in supplemented groups than in the controls (A or B) and in non supplemented groups. Thereafter the increase in lutein concentrations of livers is continuous in supplemented groups.

In case of blood and liver lycopene concentrations there is an elevation on the 5th days of the supplementation. As in the serum also in the liver the direct application of the DSM product, microcapsulated lycopene formula is more favourable (p<0.05) than the mixing this product in the egg yolk, and this form probably also earlier transported into the livers from the serum. The joint administration of these two carotenoids in the egg yolk powder resulted probably competition between the lutein and lycopene; therefore this procedure is not recommended.

Functional or designer foods and their roles in human diet have received substantial attention in recent years. The egg has a great unexplored potential in terms of improvement of human diet. As a whole food, eggs are excellent, inexpensive and low calorie sources of many favourable nutrients.

Due to both their high bioavailability and not being subject to seasonal variation, the consumption of eggs is a favourable source of lutein and zeaxanthin in the diet. One egg yolk provides approximately 200 micrograms of lutein, and lutein in eggs is 200-300 percentage more bioavailable than from vegetable sources. The lipid matrix provides an effective vehicle for increased and site-specific antioxidant uptake.

The nutrient content of the eggs can be modified by manipulating the diet of the laying hen and it is possible to produce eggs that have added health and nutritional benefits. Commercially, it is possible to produce designer eggs enriched with many different nutrients simultaneously or with a single nutrient depending on the consumer demand.

Dried and powdered egg yolk is excellent for cooking and baking in large scale in household kitchens too. The egg yolk powder is consistent and completely natural, conveniently supplied and easily stored. The dried egg yolk is pasteurized therefore it is very stable for long periods of time. Beside cookery possibilities the egg yolk powder capable as an easy to use nutraceuticum. It contains as a matter of course lutein and zeaxanthin in substantial amounts. The egg yolk powder can be easily designed during the processing. For example before the pulverization such biological active material can be also added which is not present in the raw egg (e.g. lycopene). Considering these facts the regular consumption of egg yolk powder offers protection against AMD.

Conclusions

According to our results the fortified egg yolk powder has more effective bioavailability than the pure chemical formula. It was confirmed by the feeding test in mice. In the case of lycopene supplementation the chemical formula seems to be more effective than lycopene added in yolk matrix. Maybe this was resulted because of the different polarity and solubility manner of these carotenoids. Because of the possible interaction of these two carotenoids their joint use for the fortification of egg yolk however is not recommended.

Literature


