

## **INCREASE OF THE LUTEIN CONTENT IN HENS' EGGS**

*Grčević M.\*<sup>1</sup>, Kralik Z.<sup>1</sup>, Kralik G.<sup>1</sup>, Radišić Ž.<sup>1</sup>, Mahmutović H.<sup>2</sup>*

<sup>1</sup>University of Osijek, Faculty of Agriculture, K. P. Svačića 1d, 31000 Osijek, Croatia

<sup>2</sup>BosPer Association, Bukinje bb., 75000 Tuzla, Bosnia and Herzegovina

\*Corresponding author: mgrcevic@pfos.hr

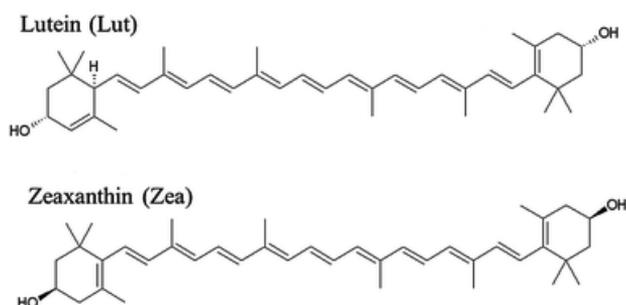
### **Abstract**

Lutein is a plant pigment that belongs to the xanthophyll group of carotenoids. In nature it is widespread (prevalent) in green leafy vegetables (spinach, kale, collard greens, lettuce) but also in peppers, tangerines, corn and egg yolk. Since it cannot be synthesized in the body it has to be taken with food. In human body lutein is concentrated in the retina and macula lutea and its content in those tissues rises with increased intake through food or supplements. It has antioxidant activity, protects eyes from high-energy blue light and helps in reducing the risk of developing age-related macular degeneration and cataracts. Until now, lutein was used in poultry industry mostly for pigmenting broiler's meat and skin and egg yolk and lately there have been more studies whose goal is to increase lutein content in yolk and production of enriched, functional food. Although table egg is not the best source of lutein, studies have shown that its bioavailability in human body is higher from lipid matrix of yolk compared with lutein from vegetable sources or food supplements. The egg is highly nutritious food because it contains high-quality proteins with balanced amino acid composition, essential fatty acids, minerals and vitamins necessary for proper functioning of the body and with increased content of lutein it becomes a value-added product. Addition of natural or synthetic sources of lutein in mixtures for laying hens enables the transfer of lutein through hen's metabolism into egg yolk. The increase of lutein content in yolk is noticeable already after one week of feeding the hens with modified mixtures although it takes a longer time for its content to be stabilized. Egg with increased content of lutein in yolk represents quality and accessible source of lutein in human nutrition. Consumption of enriched eggs contributes to increased intake of lutein as well as its accumulation in the human organism.

**Key words:** *hen's egg, lutein, yolk*

### **Introduction**

Lutein is a plant pigment which together with its stereoisomer zeaxanthin belongs to the xanthophyll group of carotenoids. Carotenoids include more than 600 compounds which are divided into two basic groups: xanthophylls (oxygen-containing molecule) and carotenes (pure hydrocarbons, without oxygen in the molecule). Unlike other carotenoids, xanthophylls contain two hydroxyl groups, one at each end of the molecule, which results in their higher polarity in comparison to other carotenoids (Golzar Adabi et al., 2010). Like other carotenoids lutein is soluble in fats and is installed in the intestine in chylomicrons that carry it to the liver. In blood it is transmitted by low (LDL) and high (HDL) density lipoproteins in equal proportions (Parker, 1996).



**Picture 1.** *Lutein and zeaxanthin*

(<http://www.rsc.org/ej/CP/2012/c2cp41583f/c2cp41583f-f1.gif>)

The human body cannot synthesize carotenoids so they should be taken with food. Lutein and zeaxanthin are most abundant in dark green leafy vegetables such as spinach, kale, collard greens, lettuce, but also in peppers, tangerines, corn and egg yolk (Sommerburg et al., 1998).

The average content of lutein in egg yolks on the Croatian market is shown in Table 1 (own research, unpublished results).

**Table 1.** *Lutein content in eggs on the Croatian market*

Lutein content	Free range eggs	Cage eggs (producer 1)	Cage eggs (producer 2)
<b>µg/g yolk</b>	35.1	15.5	12.5
<b>mg/100 g yolk</b>	3.57	1.54	1.7
<b>mg/60 g egg</b>	0.55	0.24	0.20

What makes lutein and zeaxanthin unique compared to other carotenoids in the human organism is their presence in specific eye tissues. They are highly concentrated in yellow spot (macula lutea), a small area of the retina responsible for central vision and visual acuity and the only carotenoids present in this tissue (Landrum and Bone, 2001). Together they make a pigment of the yellow spot. Due to the specific presence in eye tissues they probably have a similar role in humans like in plants, where they act as antioxidants and filters of high-energy blue light (Krinsky, 2002). In recent years there has been more intense research on the role of lutein in maintaining eye health. Lutein intake through food or food supplements increases its content in the serum of humans as well as macular pigment density which is inversely related to the risk of eye diseases, like age-related macular degeneration (Seddon et al., 1994, Bone et al., 2001; Mares-Perlman et al., 2002), a major cause of blindness in people over 65 years, and cataracts (Brown et al., 1999, Chasan-Taber et al., 1999, Gale et al., 2001). Advanced age-related macular degeneration often leads to irreversible blindness for which there is still no effective treatment (Fine et al., 2000). The role of lutein in reducing the risk of developing cardiovascular disease

(Iribarren et al., 1997, Dwyer et al., 2001; Alves-Rodrigues and Thomas, 2002), as well as its ability to protect the skin from damage caused by UV radiation (Chen et al., 2002, Stahl and Sies, 2002) is also being explored. Till now, in poultry industry, lutein was mainly used as a coloring agent for skin, meat and egg yolks, but now the emphasis is placed on increasing the content of lutein in egg yolk and the production of functional foods. Although egg yolk is not the best dietary source of lutein, its bioavailability in the human body is higher in egg yolk compared to vegetable sources or dietary supplements (Chung et al., 2004). It is assumed that the contents of cholesterol and fatty acid composition of egg yolk affect the increase of lutein in human serum (Chung et al., 2004). Therefore, the aim of this paper is to present an overview of the possibilities for enriching hen eggs with lutein, and production of functional foods which could provide multiple benefits for people consuming it.

### **Enrichment of eggs with lutein**

Eggs can be enriched with lutein by the addition of lutein sources in mixtures for hens. Two studies on the effectiveness of the transfer of lutein from hens feeds- into egg conducted Leeson and Caston (2004). In the first study hens were fed diet based on corn and soybean in which lutein was added in proportions of 0, 125, 250, 375, 500, 625, 750 and 1000 ppm. In second study hens were fed with three different mixtures. The first mixture was based on corn and soybean, comparable with the mixture in the first study. The second mixture included corn gluten and alfalfa and added corn oil instead of animal/vegetable fats. The third mixture contained ground flaxseed. Lutein was added in mixtures in proportions of 0, 125, 250 and 500 ppm. Within the first study, with an increase in lutein content in the mixture the content of lutein in eggs was also increased ( $P < 0.01$ ), although the efficiency of transfer of lutein from a mixture into eggs decreased with increasing amounts of lutein in food. The most notable increase of lutein content in eggs occurred already with the initial addition of 125 ppm of lutein in the mixture, while the maximum level of enrichment was achieved by the addition of 500 ppm of lutein. The content of lutein in egg increased from 0.16 mg/60 g of egg (0 ppm) to 1.17 mg/60 g of egg for 125 ppm of lutein in feed, and to 1.49 mg/60 g of egg with the addition of 500 ppm of lutein in feed. The results of another study showed that the addition of corn gluten and alfalfa favored disposal of lutein in eggs, while the inclusion of flax seed in a mixture partially reduced deposition of lutein. Authors believe this is troubling because of the increasing emphasis on the production of eggs at the same time enriched with various functional ingredients. Analysis of eggs showed an increase in the content of lutein from 0.18 mg/60 g of egg (0 ppm) to 1.65 mg/60 g of egg with the addition of 500 ppm of lutein in the group with a mixture based on soya and corn. The inclusion of corn gluten and alfalfa in the mixture increased the lutein levels from 0.29 mg/60 g of egg to 2.04 mg/60 g of egg (500 ppm). In the group of hens where flax seed was added to the mixture, an increase in the content of lutein from 0.24 mg/60 g of egg (0 ppm) to 1.39 mg/60 g of egg (500 ppm) was also observed although the enrichment was lower compared to the two previous groups. The authors conclude that it is possible to increase the content of lutein in eggs 5-8 times above normal values and that such enriched eggs can contribute significantly to the human diet. Leeson et al. (2007) examined the impact of long-term addition of lutein in combination with ground flax seed supplementation on disposal of lutein in eggs and tissues. Laying hens were fed mixtures with 0 or 10% of ground flax seed and three levels of lutein (0, 125 and 250 ppm) for 11 consecutive 28-day period. Addition of lutein to the mixtures significantly increased the content of lutein in eggs ( $P < 0.01$ ). Lutein content in eggs was linearly increased in the group of hens fed with 10% of

linseed (114, 1385 to 1727  $\mu\text{g}/\text{egg}$  for 0, 125 and 250 ppm of lutein in feed), whereas in the group without linseed the maximum was observed with the addition of 125 ppm of lutein in feeds (90, 1671 and 1610  $\mu\text{g}/\text{egg}$  for 0, 125 and 250 ppm of lutein in feeds), with no further increase in the lutein content in eggs. The authors were able to increase the content of lutein in eggs from 0.10 mg to 1.60 mg/60 g of egg, which represented almost two-fold increase compared to the current lutein intake in the population of North America. Golzar-Adabi et al. (2010) also investigated the effect of lutein supplemented to mixtures for laying hens on the content of lutein in eggs. To the mixtures based on corn and soybean 0, 250, 500 and 750 ppm of lutein was added. The content of lutein in egg yolks increased significantly after 7 days of feeding ( $P < 0.01$ ). The most significant increase was recorded with 250 ppm lutein supplementation, from 0.12 mg/57 g of egg to 1.35 mg/57 g of egg, while the highest content of lutein in egg yolks was observed for 750 ppm lutein added (1.43 mg/57 g egg) to the feed. Results of all the above authors show that feeding hens with lutein enriched mixtures can increase the content of lutein in egg yolk, although the enrichment was slowed with the addition of more than 250-375 ppm of lutein in feed, and it is also necessary to consider the sources of oils/fats in hens feed. Hammershoj et al. (2010) investigated the effect of the addition of three colored (orange, yellow and purple) varieties of carrots in the amount of 70 g/day/hen to the standard feed at the disposal of carotenoids in egg and some indicators of egg quality. Feeding lasted for 28 days. Lutein intake was highest with the addition of purple carrots, reaching a level of 0.8 mg/day/hen. The authors concluded that the use of colored carrot varieties as forage material in feeding hens increases the concentration of total carotenoids in egg yolks by 25-75% compared to eggs laid by hens not fed with carrots. Looking at individual carotenoids in egg yolk it is evident that the content of lutein was increased by 54% and that of  $\beta$ -carotene 100 times. Purple carrot cultivar contained the highest concentration of both lutein and  $\beta$ -carotene and therefore had the most significant impact on the content of carotenoids in egg yolks. Jeon et al. (2012) conducted a trial in which hens were fed mixtures supplemented with green algae *Chlorella* powder in proportions of 0, 0.5, 1 or 2%. Feeding with experimental mixtures lasted for 4 weeks. Already after first week of feeding authors have observed an increase in the content of lutein in egg yolks in all experimental groups compared to the control. After four weeks of feeding in the group with 2% *Chlorella* powder the highest content of lutein was recorded and it amounted to 27.04  $\mu\text{g}/\text{g}$  of egg yolk compared to 13.88  $\mu\text{g}/\text{g}$  in the control group. The authors concluded that for the production of lutein-enriched egg it takes at least two to three weeks of feeding with *Chlorella* powder to ensure the highest possible incorporation of lutein in egg yolk. Jang et al. (2014) fed hens during five weeks with control mixture and mixtures to which a commercial lutein (40 mg/kg) and the crude extract of spinach dissolved in the oils with lecithin, in which the concentration of lutein was also 40 mg/kg, were added. The content of lutein increased in both experimental groups. In group with commercial lutein there was approximately four times more lutein than in the control group. Although there were no statistically significant differences between the experimental groups, in the group with commercial lutein higher content of lutein and less variability in the average content of lutein in egg yolks than in the group with spinach extract was recorded. These results suggest that commercial lutein provides a more efficient and more uniform transmission of lutein in egg yolks than spinach extract in laying hens.

## **Conclusion**

Chicken egg is a foodstuff that provides the highest quality proteins, essential amino and fatty acids, vitamins and minerals in optimal quantities for the human body. Fatty acid

composition of egg yolk as well as the content of vitamins and other fat-soluble substances can be altered through hens nutrition. The content of lutein also. Lutein is a pigment that protects plant cells from oxidation and the harmful effects of solar radiation. Apart from the plant world, lutein shows a protective role in other systems also, such as the human body, which is confirmed by various *in vitro* and *in vivo* studies. It is significant for protection of eyes from developing age-related macular degeneration, and its role in protecting the skin and circulatory system is also explored. Because it is soluble in fats its content in egg yolk may be affected by the addition of lutein sources in mixtures for laying hens. Various authors in their researches showed that it is possible to successfully increase the content of lutein in egg by adding various amounts of natural or synthetic sources of lutein in hens' feed, wherein for synthetic lutein more uniform and prolonged egg yolk enrichment was recorded. Lutein-enriched egg, in addition to the above nutritional quality, has added value in terms of functional activity. Consumption of eggs enriched with lutein increases the content of lutein in human plasma as well as the density of yellow spot pigment, thereby contributing to better protection of the eyes and stronger antioxidant protection of the organism.

## References

1. Alves-Rodrigues A and Thomas B 2002. The role of lutein in the prevention of atherosclerosis. *J. Am. Coll. Cardiol.* 40, 835–836.
2. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE and Twaroska EE 2001. Macular pigment in donor eyes with and without AMD: A Case–Control Study. *Invest. Ophthalmol. Vis. Sci.*, 42, 235-240.
3. Brown L, Rimm EB, Seddon JM, Giovannucci EL., Chasan-Taber L, Spiegelman D, Willett WC and Hankinson SE 1999. A prospective study of carotenoid intake and risk of cataract extraction in US men. *Am. J. Clin. Nutr.* 70, 517-524.
4. Chasan-Taber L, Willett WC, Seddon JM, Stampfer MJ, Rosner B, Colditz GA, Speizer FE and Hankinson SE 1999. A prospective study of carotenoid and Vitamin A intakes and risk of cataract extraction in US women. *Am. J. Clin. Nutr.* 70, 509-516.
5. Chen J, Wu A, Pathak MA, Rius-Diaz F, Mihm CM, Goukassian D and Gonzalez S 2002. Dietary lutein and zeaxanthin partially prevent UVB-induced skin careinogenesis in the SKh-1 hairless mouse model (Abstract). In: Society for Investigative Dermatology. Los Angeles, CA.
6. Chung HY, Rasmussen HM and Johnson EJ 2004. Lutein bioavailability is higher from lutein-enriched eggs than from supplements and spinach in men. *J Nutr.* 134, 1887-1893.
7. Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, Shircore A, Hama-Levy S, Hough G, Wang X, Drake T, Merx CN and Fogelman AM 2001. Oxygenated carotenoid lutein and progression of early atherosclerosis: The Los Angeles Atherosclerosis Study. *Circulation* 103, 2922–2927.
8. Fine SL, Berger JW, Maguire MG and Ho AC 2000. Age-related macular degeneration. *N. Engl. J. Med.* 342, 483-492
9. Gale CR, Hall NF, Phillips DI and Martyn CN 2001. Plasma antioxidant vitamins and carotenoids and age-related cataract. *Ophthalmology* 108, 1992-1998.
10. Golzar Adabi SH, Kamali MA, Davoudi J, Cooper RG and Hajbabaei A 2010. 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. *Arch.Geflügelk.* 74, 3, 158-163.

11. Hammershøj M, Kidmose U and Steinfeldt S 2010. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (*Daucus carota*) varieties as forage material for egg-laying hens. *J. Sci. Food. Agric.* 90, 1163-1171.
12. Hamulka J, Koczara J and Gronek M 2005. Lutein content of selected polish foods and estimation of its intake. *Pol. J. Food Nutr. Sci.* 14, 2, 201–206.
13. Iribarren C, Folsom A, Jacobs JrD, Gross MD, Belcher JD and Eckfeldt JH 1997. Association of serum vitamin levels, LDL susceptibility to oxidation and autoantibodies against MDA-LDL with carotid atherosclerosis A case-control study. The ARIC Study Investigators. *Atherosclerosis Risk in Communities. Arterioscler. Thromb. Vasc. Biol.* 17, 1171–1177.
14. Jang I, Ko Y, Kang S, Kim S, Song M, Cho K, Ham J and Sohn S 2014. Effects of Dietary Lutein Sources on Lutein-Enriched Egg Production and Hepatic Antioxidant System in Laying Hens. *J. Poult. Sci.* 51, 58-65, 2014
15. Jeon JY, Kim KE, Im HJ, Oh ST, Lim SU, Kwon HS, Moon BH, Kim JM, An BK and Kang CW 2012. The Production of Lutein-Enriched Eggs with Dietary *Chlorella*. *Korean J. Food Sci. Ani. Resour.* 32, 1, 13-17.
16. Krinsky NI 2002. Possible biologic mechanisms for a protective role of xanthophylls. *J. Nutr.* 132, 540-542.
17. Krinsky NI, Landrum JT and Bone RA 2003. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu. Rev. Nutr.* 23, 171-201.
18. Landrum JT and Bone RA 2001. Lutein, zeaxanthin, and the macular pigment. *Arch. Biochem. Biophys.* 385, 1, 28-40.
19. Leeson S, Caston L and Namkung H 2007. Effect of dietary lutein and flax on performance, egg composition and liver status of laying hens. *Can. J. Anim. Sci.* 87, 365-372.
20. Leeson S and Caston L 2004. Enrichment of eggs with lutein. *Poult. Sci.* 83, 1709-1712.
21. Mares-Perlman JA, Millen AE, Ficek TL and Hankinson SE 2002. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. *J. Nutr.* 132, 3, 518-524.
22. Parker RS 1996. Absorption, metabolism, and transport of carotenoids. *FASEB J.* 10, 5, 542-51.
23. Perry A, Rasmussen H and Johnson EJ 2009. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. *J. Food Comp. Anal.* 22, 9-15.
24. Seddon JM, Ajani UA, Sperduto FLD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT, Yannuzzi LA and Willett W 1994. Dietary carotenoids, Vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA.* 272, 1413-1420.
25. Sommerburg O, Keunen JE, Bird AC and van Kuijk FJ 1998. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Br J Ophthalmol.* 82, 8, 907-10.
26. Stahl W and Sies H 2002. Carotenoids and protection against solar UV radiation. *Skin Pharmacol. Appl. Skin Physiol.* 15, 291–296.
27. <http://www.rsc.org/ej/CP/2012/c2cp41583f/c2cp41583f-fl.gif>