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REGULAR ARTICLE

EXPERIMENTS OF FORTIFIED EGG YOLK POWDER IN MICE

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ABSTRACT

The egg contains inorganic binding nutrients, which are essential in human feeding. Besides a great number of nutrients, vitamins and microelements can be accumulated in egg yolk. The enrichment of vitamins and carotinoids in egg yolk can be achieved by addition of desired supplementary compounds to the feed. Fortified egg yolk can be a good source of different carotenoids, and vitamin C. Carotenoids have protecting effect as follows: antioxidant activity, immunostimulation effect and antimutagenous effect.

The ascorbic acid's biological effect is ability of oxidation-reduction. It has reduction capacity, and takes part in regeneration after glutation oxidation.

In our experiment BALBc mice were fed by fortified egg yolk powder. Carotenoid, lutein, lycopene, and vitamin C were mixed into the egg yolk before lyophilisation (lutein 0.014 %; vitamin C 0.035 %, likopin 0.035 %) in food. The other mice group was fed with syntetic form of lutein, lycopene and vitamin C. Mice were bled at 5th and 10th day of experiment. Blood and liver concentrations of lutein, lycopene and vitamin C were measured by HPLC method. In our experiment we compared utilization of lutein, lycopene and vitamin C concentration in different fortified food of BALBc mice.

Keywords: lutein, lycopene, vitamin C, egg yolk powder, mouse

INTRODUCTION

Eggs contains most of recognized vitamins the exception of vitamin C. The egg is a source of vitamin B. It is particularly rich source of vitamins B12 and riboflavin and a useful source of folate. The egg is also a good source of the fat-soluble vitamins A and D and provides some vitamin E. Eggs contains many of the minerals that the human body requires for health. In particular eggs are an excellent source of iodine, required to make the thyroid hormone, and phosphorus, required for bone health. The egg is a significant source of selenium, an important antioxidant and provides iron and zinc **(Layman and Rodrigez 2009).**

Ascorbic acid is a naturally occurring organic compound with antioxidant properties. It dissolves well in water, to give mildly acidic solutions. Ascorbic acid is one form of vitamin C. As a mild reducing agent, ascorbic acid degrades upon exposure to air, converting the oxygen to water. The redox reaction is accelerated by the presence of metal ions and light. Ascorbate usually acts as antioxidant. It is typically, reacts with oxidants of the reactive oxygen species, such as the hydroxyl radical formed from hydrogen peroxide **(Bendich, 1992).** Such radicals are damaging to animals and plants at the molecular level due to their possible interaction with nucleic acid, proteins and lipids. Vitamin C is known to inhibit the conversion to nitrosamines in stomach **(Stocker and Frei, 1991).**

Lycopene is a bright red carotene and carotenoid pigment and phytochemical found in tomatoes and other red fruits and vegetables such as watermelons, papayas. It has no vitamin A activity.

The main biological effects of carotenoids are discussed in detail in a review article of our work groups **(Bárdos et al., 2011)** and present experiment is continuing of our research work from last year.

MATERIAL AND METHODS

Feed additives

The experimental feed was supplemented with lutein, lycopene and vitamin C: either with egg yolk powder containing these chemicals or with gelatine coated formulations (Lutein 5 % CWS/S-TG; Redivivo^{TM} lycopene 5 % CWS/S both DSM, Nutritional Products Ltd. Basel, Switzerland) and ascorbic acid 99 % (Carl Roth GmbH Germany) in parallel.

Preparation of fortified egg yolk powder

Commercial eggs were broken and an aliquot volume of yolk was mixed with lutein, with lycopene, with vitamin C and with all 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. Nine 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 an enriched feed. Basal diet used was laboratory mice feed. We mixed the additives with it. After grinding this feed was mixed with either antioxidant containing egg yolk powder or microcapsulated preparates hereby the final concentrations were 0.035 % (w/w) related to feed respectively. From the mixtures we formed scones using cooking gelatine so we were able to apply them for feeding after dehydration **(Bárdos and Bender 2012)**. Control diets were formulated too. It was prepared without any supplementation. The animals were fed for 10 days, contains the experimental and feeding set-up. Five mice from each group were picked out and *lege artis* sacrificed on the 5^{th} and 10^{th} days after the beginning of dietary supplementations. Blood sera and liver were obtained for chemical analyses.

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 in brief: the samples were extracted with extraction mixture (hexane: acetone: ethanol: toluol 10:7:6:7) and saponified in alcoholic potassium hydroxide at 56°C for half an hour. The extract got cold at room temperature in dark for one hour and was mixed with hexane again. After the solution was placed in darkness for 1 hour again 400 µl of supernatant was evaporated in N_2 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) which was connected to the HPLC system what consisted of PU-980 pump and 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 for lutein and 505 nm for lycopene. Identification of anilities based on standards and the concentrations were calculated by Chrompass software (Jasco, Japan).

Statistical analysis

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

RESULTS AND DISCUSSION

The results are presented in figures. The blood lutein and lycopene are shown in the Figure 1 – 8, and the liver carotenoid concentration in the Figure 5 and 6.

Lutein supplementations in egg yolk powder resulted higher lutein concentrations as 5th day as 10th day. Similar results after lycopene supplementation were detected.

Lycopene is not an essential nutrient for humans, but it is commonly found in the diet mainly from dishes prepared from tomatoes. When absorbed from the stomach, lycopene is transported in the blood by various lipoproteins and accumulates in the liver, adrenal, gland, and testes. Because preliminary research has shown on inverse correlation between consumption of tomatoes and cancer risk lycopene has been considered a potential agent for prevention of some types of cancers particularly prostate cancer **(Florence 1990).**

Human and animal investigations have demonstrated that carotenoids are associated with a number of health benefits **(Rao and Rao, 2007).**

These benefits of carotenoids are antioxidant activities, anti tumor effects, positive immunomodulations. One of the most investigated roles of carotenoids is the preventive and curative effects 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 of retina. The macula lutea contains oxycarotenoids (lutein and zeaxanthin) in significant amounts. 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. Eating a well-balanced diet with plenty of fresh fruits and vegetables containing oxycarotenoids (lutein and zeaxanthin) and stopping smoking may all help to delay the progress of AMD. Besides the green vegetables the egg yolk also contains lutein and zeaxanthin in high amounts, too. The lutein bioavailability from egg is higher than that from other vegetable sources.

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.

Our results showed that the lycopene and lutein concentration in blood and liver reached the highest level, when the food was supplemented with the combination by vitamin C. Lengyel et al (2004) have detected highest microelement concentration which were given the additives vitamin C.

Based on our results the conclusion can be drawn, the lutein and lycopene compound increased the level in blood and liver too, especially together the vitamin C in mice.

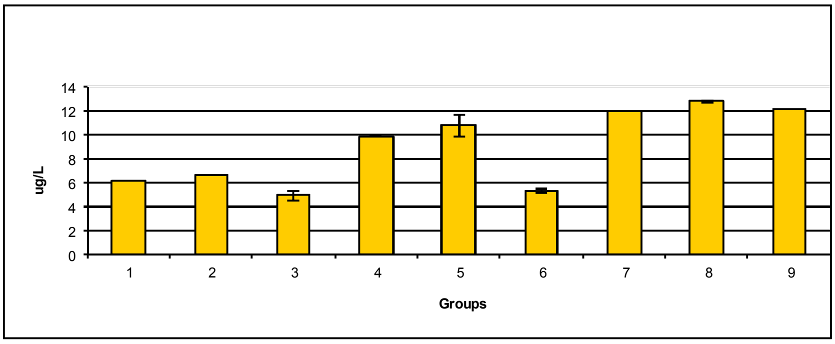

Figure 1 Lutein concentration in mice blood on 5th day

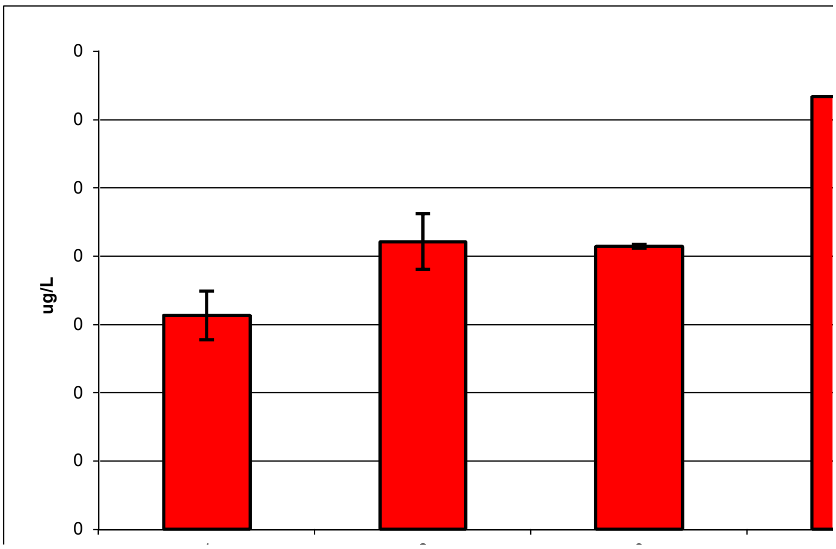

Figure 2 Lutein concentration in mice blood on 10th day

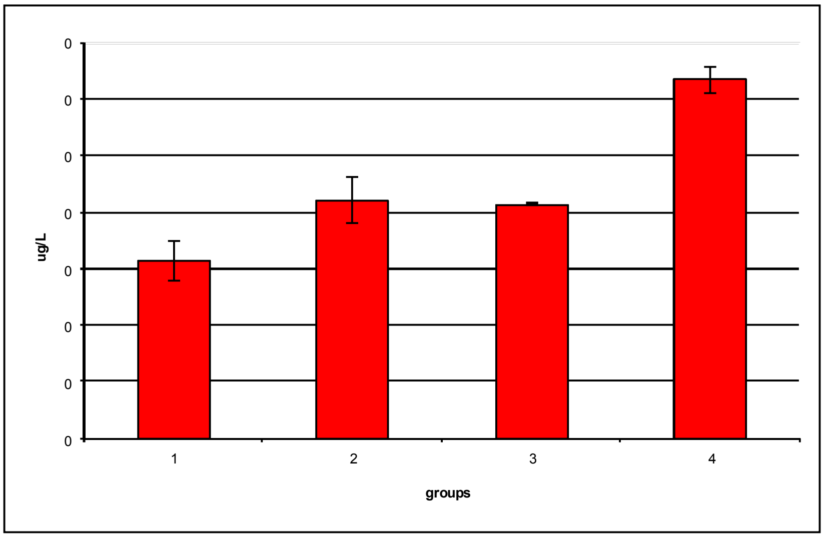

Figure 3 Lycopene concentration in mice blood on 5th day

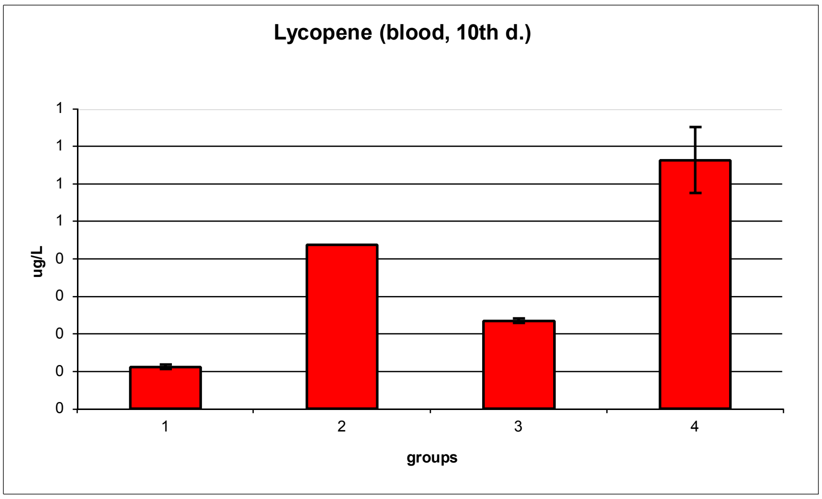

Figure 4 Lycopene concentration in mice blood on 10 th day

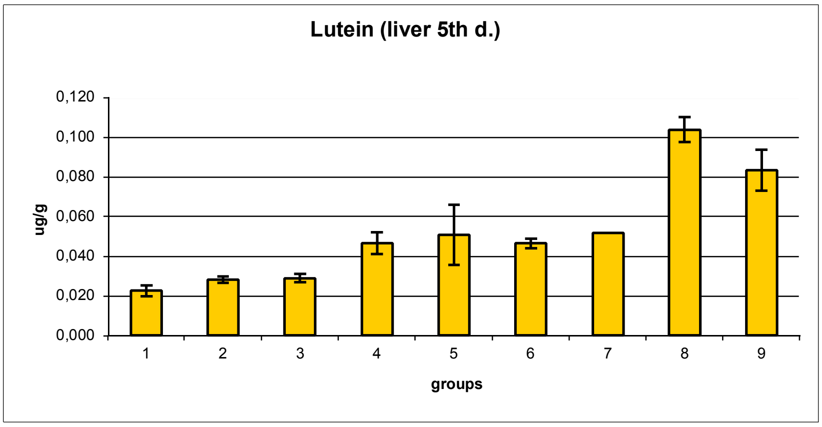

Figure 5 Lutein concentration in mouse liver on 5th day

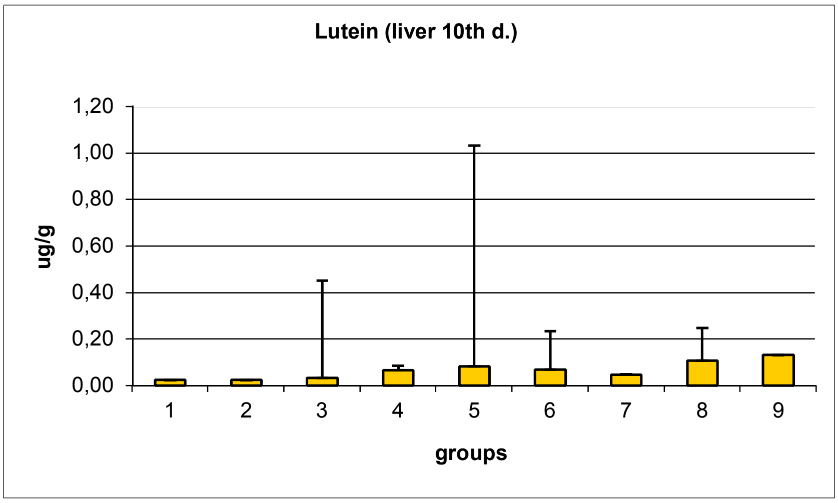

Figure 6 Lutein concentration in mouse liver on 10th day

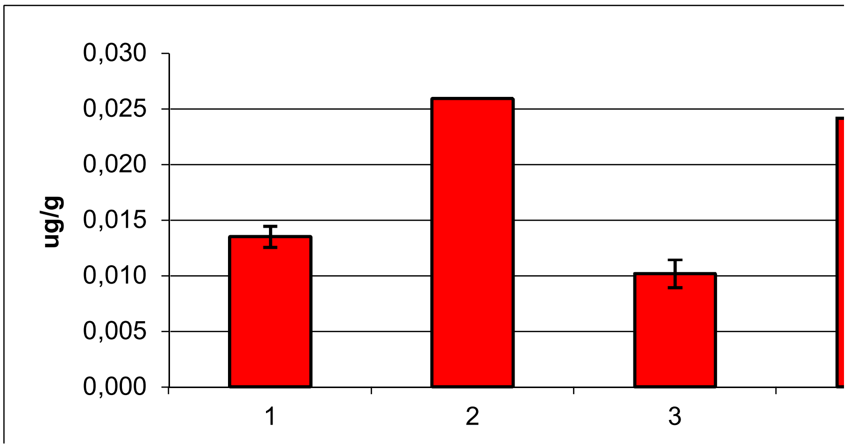

Figure 7 Lycopene concentration in mouse liver on 5th day

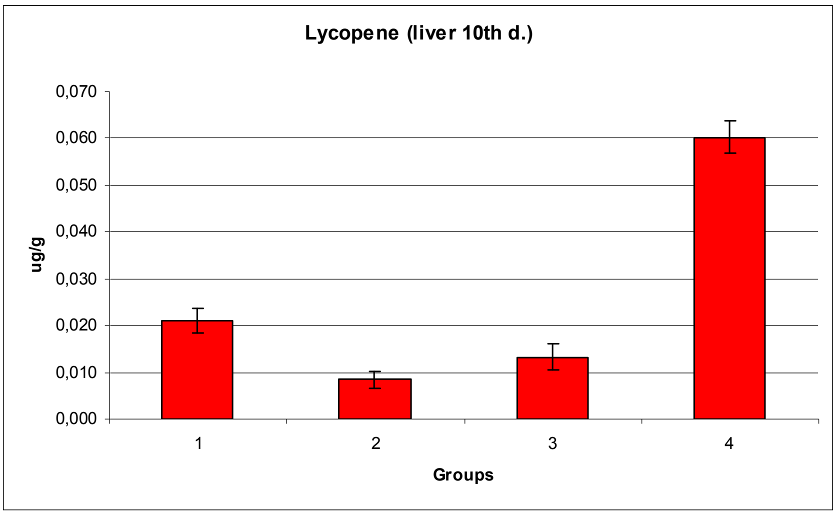

Figure 8 Lycopene concentration in mouse liver 10th day

REFERENCES

BÁRDOS, L. – BENDER, B. 2012. Effect of apple cider vinegar on plasma lipids (Model experiment in mice). In *Potravinárstvo*, vol. 6, 2012, p. 1-4.

BÁRDOS, L. – JUNG, I. – KERTI, A. – SZABÓ, CS. – KISS, ZS. – LAKNER, H. 2011. Carotenoids and body defense in accordance with experiences in poultry. In Risk Factors and Biological Systems Vol I. eds.: LUKÁČ, N. – MASSÁNYI, P. Slovak University of Agriculture in Nitra, pp. 114-124.

BENDICH, A. 1992. The role of carotenoids in immune response. In *Stichting Voedingscentrum Nederland*, vol. 53, 1992, p. 191-195.

FLORENCE, T. M. 1990. Free radicals, antioxidants and cancer prevention. In *Proceedings of the Nutrition Society of Australia*, vol. 15, 1990, p. 88-93.

KERTI, A. – BÁRDOS, L. 2006. Simultaneous determination of retinoids (retinol, retinyl palmitate) and carotenoids (lutein, zeaxanthin, ß-cryptoxanthin, lycopene, ß-carotene) and vitamin E by RP- HPLC. In *Clinical and Experimental Laboratory Medicine*, vol. 32, 2006, p. 106.

LAYMAN, K.L. – RODRIGEZ, N.R. 2009. Egg protein as a source of power, strength and energy. In *Nutrition Today*, vol. 44, 2009, p. 1.

LENGYEL, L. – SZABÓ, M. – BÁRDOS, L. – KISS, ZS. 2004. Megnövelt antioxidáns anyag tartalmú tojás előállítása és annak hasznosulás egerekben.-Szelén az élettelen és élő természetben ”Kerekasztal Konferencia SZIE Gödöllő. Kisállattenyésztési-és Takarmányozási Kutatóintézet, 2004, pp. 213..

RAO, A.V. – RAO, G.L. 2007. Carotenoids and human health. In *Pharmacological Research*, vol. 55, 2007, p. 207-216.

SEDDON, J.M. – AJANI, U.A. – SPERDUTO, R.D. – HILLER, R. – BLAIN, N. – BURTON, T.C. – FARBER, M.D. – GRAGOUDOS, E.S. – HALLER, J. – MILLER, D.T. – YANNUZZI, L.A. – WILLETT, W. 1994. Dietary carotenoids, vitamin A, C, and E, and advanced age-related macular degeneration. In *Journal of American Medical Associtiion*, vol. 272, 1994, p. 1413-1414.

STOCKER, R. – FREI, B. 1991. *Oxidative stress: Oxidant and antioxidants*. Academic Press, London, pp. 213-243.