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FUNCTIONAL EGG PRODUCTION. III. THE ROLE OF THE CAROTENOIDS

(review)

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Abstract

Recent growth of public awareness on the role of carotenoids (especially xanthophylls) in the prophylaxis and dietotherapy of certain oncologic, cardiovascular, and ocular diseases in human related to antioxidative and immunomodulating properties (E. Bakan et al., 2014) made these substances used earlier for the improvement of egg yolk color (and, as a consequence, attractibility of the eggs for consumers) valuable target substances in the production of dietetic, designer, and functional eggs (V.P. Singh et al., 2012). High bioavailability of carotenoids from table eggs due to the solubilization in yolk lipids makes the eggs a suitable source of carotenoids for the enrichment of human diet (H.-Y. Chung et al., 2004). In the study presented different aspects of practical production of functional eggs enriched with carotenoids are reviewed: the sources of carotenoids in the diets for laying hens and their comparative efficacy; metabolism of carotenoids in hens and its relation to the metabolism of lipids; the effects of different xanthophyll sources on health and productivity in hens, egg quality, the intensity of yolk coloration, concentrations of xanthophylls in yolk. The main advantage of synthetic preparations of carotenoids is their high availability for the layers (M. Marounek et al., 2016); however, due to their expensiveness these sources can be economically unprofitable in the production of enriched eggs. Natural sources of basic yolk carotenoids, lutein and zeaxanthin (S.M. Vostrikova et al., 2011), e.g. marigold (*Tagetes* spp.), frequently contain substantial part of these xanthophylls as ethers with fatty acids; the availability of these forms for layers is significantly lower in compare to the saponified forms (K. Lokaewmanee et al., 2011). These sources can be preliminary saponified to release the etherified xanthophylls: it will improve the availability of the latter for layers by 40-60 %, the transfer of these substances to egg yolks (H. Hencken, 1992), and prevent the resulting fatty acid profiles of eggs from the shift to higher percentage of saturated fatty acids (A. Altunta et al., 2014). The disadvantage of this approach is related to low stability of saponified preparations during the storage; researchers recommend these preparations to be stored frozen and to be used as soon as possible after the unpacking. Different effects of the sources of xanthophylls on the productive performance in layers (primarily the intensity of lay, egg weight and morphology) were reported: certain authors reported the significant improvements of these productivity parameters while other authors found these parameters to remain at the level of control treatments or be slightly lower. The majority of studied sources providing xanthophylls in the forms available for layers substantially and significantly improves the parameters of yolk coloration intensity (decreases index of lightness L* and increases indices of yellowness b* and redness a*) and concentrations of lutein and zeaxanthin in yolk. Reasonable choice of sources and doses can result in eggs enriched with xanthophylls to the extent where the eggs could be considered functional carotenoid sources for human (A. Sahoo et al., 2014), though no normal consumption rates for the xanthophylls irrespective to specific diseases were so far developed (E. Toti et al., 2018).

Keywords: functional eggs, carotenoids, lutein, zeaxanthin, layer diet, dietary carotenoid

sources, egg yolk quality

In previous reports, the authors discussed the issues related to the production of ω-3 polyunsaturated fatty acid [1], selenium, zinc and iodine [2] functional hen eggs, as well as the enrichment (biofortification) of eggs with vitamins and carotenoids [3]. In this part of the reports series, special attention is paid to the production of carotenoid-functional eggs, as information is currently being accumulated on these substances as a means of preventing a number of human diseases.

Chemically, carotenoids are tetraterpene pigments with a color ranging from pale yellow to dark red [4], which are synthesized by many microorganisms and plants, as well as some algae and fungi. The animal organism is not capable of synthesizing de novo carotenoids [5]. More than 750 of these compounds are already known, and this list is updated every year [6]. They can form esters with fatty acids and complexes with sugars and proteins [7]. Some of them, for example, β- and γ-carotenes, cryptoxanthins, but not all of them, exhibit A-provitamin activity [8]. About 50 carotenoids with A-provitamin activity are known [9]. Carotenoid color is due to chains of conjugated double bonds acting as a chromophore group [10]. The same structures are responsible for the antioxidant properties of carotenoids: the more conjugated double bonds in the carotenoid molecule, the better its ability to bind the formed active oxygen species or to prevent their development [11]. The antioxidant properties of carotenoids in vivo are due to their interactions with other antioxidants, such as vitamins E and C [12].

The most important carotenoids for humans, based on the consumption with various products and plasma blood concentration, include α- and β-carotenes, lycopene (hydrocarbon carotenoids, or carotenes), lutein, zeaxanthin and β-cryptoxanthin (oxycarotenoids, or xanthophylls) [13]. In total, up to 40 different carotenoids can be present in the human diet [14]. It is known that carotenoids reduce the risk of certain types of malignant neoplasms (breast, lung, ovarian, prostate cancer), cardiovascular diseases, eye diseases (cataracts, age-related macular degeneration). The anti-cancer activity of α- and β-carotenes, lycopene, canthaxanthin and a number of other xanthophylls is associated with several possible mechanisms. Carotenoids with A-provitamin activity can affect the differentiation and division of cancer cells; antioxidant activity protects the DNA of healthy cells from damage by reactive oxygen species; the immunomodulatory effect enhances the control of carcinogenesis by the immune system; carotenoid enhancement of the intercellular signaling system inhibits the expansion of initiated cancer cells [15]. The role of carotenoids in the prevention of cardiovascular diseases is associated with their antioxidant activity: they protect low-density lipoproteins from oxidation, which contributes to the development of atherosclerosis, including coronary atherosclerosis [16]. The relationship between carotenoid intake with food and the frequency of cardiovascular diseases was confirmed by large-scale examinations of patients in Italy [17], Japan [18], EU countries [19], and Costa Rica [20].

The protective effect in eye diseases is carried out primarily by lutein and zeaxanthin, which are the only carotenoids present in the retina and lens of the eye [21]. They are part of the pigment of the so-called macula [22]. Two mechanisms have been proposed to explain the protective effect of carotenoids against age-related macular degeneration. The first is due to the fact that the macular pigment protects the photoreceptors and retinal epithelium from harmful blue light, which is well absorbed by both lutein and zeaxanthin [23]. The second mechanism is associated with the fact that the specified carotenoids reduce oxidative stress caused by light and photo-induced changes in the metabolism of this tissue [24]. The antioxidant effect also explains the decrease in the frequency of cataracts due to these carotenoids (especially lutein). Thus, an in vitro experiment showed that lutein could inhibit lipid peroxidation induced by ultraviolet light in a culture of human retinal epithelial cells [25].

The main source of carotenoids in the human and animal diet is fruits and leaves of plants. The content of carotenoids in them and the bioavailability of these substances depend on the type of plant, its maturity, time of harvest, growing and storage conditions [26]. The bioavailability of carotenoids from concentrated preparations can also vary greatly [27]. For example, about 5% of carotenoids are absorbed from whole raw vegetables in the intestine, while up to 50% are absorbed from micellar solution, therefore the physical form in which carotenoids enter the intestinal mucosa cells is extremely important [28]. The chemical form of carotenoids is no less important. It is known that in hens free lutein is absorbed much more efficiently than its mono- or diethyl ethers, in the form of which this carotenoid can be found, for example, in marigold flowers, and that preliminary hydrolysis of these esters increases the absorption of lutein by 40-60%. The same applies to esters of zeaxanthin and capsanthin, the red pepper carotenoid [29]. It has been established that in the yolk and other tissues of hens' body, lutein is deposited mainly in its free form (80%) and to a lesser extent in the form of metabolites, mono- and diesters, and 3′-oxolutein [30]. On the other hand, it should be noted that both mono- and diesters of carotenoids are also effective antioxidants, since esterification does not affect or screen their polyisoprenoid chains [31].

Since most carotenoids are fat-soluble, their absorption and metabolism are associated with the absorption of fats. After absorption in the intestine and metabolization in the liver, carotenoids are transported into the blood by lipoproteins with low (non-polar carotenoids, i.e. carotenes) or high density (xanthophylls such as lutein or zeaxanthin). Then, these complexes with lipoproteins are decomposed by lipoprotein lipases with the release of free carotenoids [32].

The effectiveness of the absorption of carotenoids is affected by the composition of the diet. Thus, some plants, in addition to carotenoids, contain inhibitors of their absorption [33]. It has also been reported that carotenoid absorption worsens when there is a significant amount of coarse fiber in the diet [34]. Another factor is the interaction between individual carotenoids: it is assumed that the introduction of a large amount of a carotenoid into the diet affects the metabolism of other carotenoids, and can both reduce and increase their absorption. It has been shown that when mixtures of xanthophylls and β-carotene are added to the diet in approximately equal proportions, the latter inhibits the absorption of canthaxanthin [35] and lutein [36], as judged by the decrease in their concentration in blood plasma.

The bioavailability of carotenoids from food eggs is higher than from plant components of the human diet [37] due to solubilization in egg yolk lipids. So, one egg enriched with carotenoids can provide 5-10% of the daily human need [38]. According to Sahoo et al. [39], the total content of lutein and zeaxanthin in high-quality food eggs is 0.4-0.5 mg, and eggs with a content of about 2.2 mg/egg are already considered to be designer, that is, a functional food product. Russian researchers reported that the concentration of carotenoids in eggs from industrial laying hens receiving standard diets was 20 μg/g yolk [40].

Since the absorption of carotenoids in hens is also associated with the metabolism of feed fats, age and fat content in the diet affect their accessibility to poultry. It is known that at an early age, the efficiency of fat digestion in hens is low; therefore, the absorption of carotenoids during this period of ontogenesis is also small, but increases with age [41]. It was reported that the introduction of an additional 6% of fat in the diet of chickens enriched with lutein leads to a 3-fold increase in its deposition in body tissues [42]. The same paper notes that some mycotoxins (aflatoxins, ochratoxin), as well as diseases (coccidiosis, Newcastle disease), reduce the efficiency of absorption and deposition of carotenoids in body tissue, especially in young animals. It should be noted that the introduction of carotene preparations into the diets of laying hens against the background of chronic mixed mycotoxicoses increased the survival rates of birds and reduced the intensity of the clinical manifestations of toxicosis [43].

The carotenoid profile of eggs is largely determined by the diet of laying hens, by varying which the carotenoid picture of eggs can also be changed directionally [44-46]. Carotenoids (e.g. canthaxanthin) are used as feed additives in industrial laying hens' diets to improve the color of the yolk; however, the amount and type of such additives may vary in accordance with the country's standards for the use of such additives and local prices [47]. It is known that xanthophylls are much more efficiently deposited in the yolk compared to carotenes [48] and to a much greater extent affect the color intensity of the yolk [49]. A low degree of transfer of carotenoids with A-provitamin activity to the yolk is due to the fact that they are mainly used by the body of the layer hen itself [50]. The most common xanthophylls in the yolk of eggs of various bird species are lutein and zeaxanthin [51, 52].

The efficiency of egg yolk pigmentation with carotenoids depends on their absorption, transport, excretion, deposition in tissue and bioconversion in laying hens, and all these factors are significantly influenced by carotenoid sources. In the study using radioactive labels, the degree of deposition of feed carotenoids into the yolk varied from 14% for astaxanthin to 30-40% for canthaxanthin [29]. The deposition of feed carotenoids into the yolk occurs within 48 hours, although the uniformity of staining is achieved later, after about 7-10 days [53].

As natural sources of carotenoids in chickens' rations, corn and its derivatives (gluten, alcohol bard) are used, including corn modified to increase the content of various carotenoids [54], tomatoes, carrots with different carotenoid picture, red pepper, flour from alfalfa, marigold flowers, marigold, carotenoid-rich algae, etc. [55]. Moreover, tomatoes, carrots and alfalfa contain more carotenes (β-carotene, lycopene) than xanthophylls [56, 57]. Synthetic carotenoids are also used, for which preparations of various compositions have been developed in recent decades [58, 59]. It should be borne in mind that not all of these sources of carotenoids are suitable for producing functional and/or dietary eggs. For example, specific red pepper carotenoids (capsanthin, capsorubin), in addition to antioxidant activity, can exhibit allergenic or toxic properties [60]. It is undesirable to use such sources (even with their low bioavailability for laying hens) when producing eggs with desired therapeutic properties.

In an experiment to investigate the effectiveness of the deposition of fodder lutein in the yolk [61], hens received lutein in doses of 0, 125, 250, 375, 500, 625, 750 and 1000 g/t with a basic corn-soy diet. With an increase in the feed lutein dose from 0 to 375 g/t, its content in eggs on the 7th day of the experiment significantly increased from 0.3 to 1.5 mg per 1 egg weighing 60 g ($p < 0.01$). The yolk coloring intensity score according to the Roche color scale increased from 6-7 to 13-14. However, with a further increase in the dose of lutein in the diet, a significant increase in its content in the eggs was not detected. It should be noted that in this experiment, the same diet supplemented with corn gluten and alfalfa flour increased the lutein content in eggs (up to 2.2 mg/egg at a lutein dose of 500 g/t), while the addition of flaxseed rather reduced this indicator. It can be assumed that the plateau of yolk saturation with lutein occurred as a result of the limited ability of the liver to metabolize feed lutein and transfer it to the yolk during vitellogenesis, and not due to the limited ability of lutein to solubilize in yolk lipids. The source of lutein in this experiment was a 5% premix based on marigold flowers, that is, fodder lutein probably contained a significant amount of lutein mono- and diesters, which were significantly less absorbed. In a later experiment [62], different doses of flour from the flowers of marigolds and a hydrolyzed extract of this flour, where the lutein esters were saponified, were compared. Each source was fed at doses of 10, 20, 30 and 40 g/t per lutein. Neither the type nor the dose of the lutein preparation had a significant effect on the main indicators of egg quality and laying productivity. An improvement in the yolk coloring indicators was noted, and it was reliable only at a dose of 40 g/t for unprocessed flour and at doses of 20 to 40 g/t for hydrolyzed extract. The deposition in the yolk of common xanthophylls (lutein + zeaxanthin) in the groups receiving the flour, and not its hydrolyzed extract, increased from 2.0 (control) to 4.3 mg/100 g (dose 40 g/t), and in the groups receiving the extract up to 5.3 mg/100 g with the same dose of feed lutein. This increase was mainly due to increased lutein deposition, while zeaxanthin deposition increased slightly.

In a similar experiment with different doses of canthaxanthin (from 0.5 to 64 g/t of feed), its relative deposition in the yolk was 40%, regardless of the dose in the diet, although the absolute deposition increased a hundredfold, from 0.026 to 2.5 mg/1 egg [63]. A highly reliable ($p < 0.001$) decrease in the indices of lightness (L^*) and yellowness (b^*) of the yolk and an increase in the redness index (a^*) were also noted. The color indices of the yolk were measured using Minolta colorimeters (Konika Minolta, Japan). These indices reflect lightness (L^*: 0 = black, 100 = white), redness (a^*: –100 = green, +100 = red) and yellowness (b^*: –100 = blue, +100 = yellow) of the yolk. The egg-laying rate of the hens remained comparable to the control. Recently, it was reported that the introduction of canthaxanthin (3 and 6 g/t) into the diet of laying hens significantly increased not only their productive indicators (egg production rate, egg weight and feed conversion), but also the strength of the vitelline membrane in the yolks of freshly delivered eggs, which again decreased during storage [64]. Although the authors considered this indicator as a characteristic of the incubation qualities of an egg, it can have a certain value in terms of egg processing technology.

In the experiment of Czech authors [65], the effects of adding lutein (250 g/t), chlorella (12.5 kg/t) and a mixture of synthetic carotenoids Carophyll red and Carophyll yellow (DSM Nutritional Products, Switzerland; 20 and 15 g/t, respectively) to laying hens' diet on yolk coloration, the oxidative stability of yolk lipids, and hens' productivity were compared. All studied additives significantly increased the egg mass (Carophyll by 2.9%, lutein by 1.6%, chlorella by 2.0%), shell weight and thickness ($p < 0.001$), and also reduced the yolk/protein ratio. At the same time, lutein, unlike other additives, significantly increased the strength of the shell. With the same significance, synthetic carotenoids and chlorella increased the color intensity of the egg yolk. The yolk redness index (a^*) was distributed among the experimental groups in the following order: control < chlorella < Carophyll < lutein; the yellowness index (b^*) increased in the groups receiving chlorella and lutein. Lutein and chlorella significantly ($p < 0.001$) increased the concentration in the yolk of lutein (from 12.8 in the control to 133.9 and 49.0 mg/kg dry matter, respectively) and zeaxanthin (from 9.2 to 123.9 and 40.1 mg/kg dry matter). All three additives significantly increased the oxidative stability of yolk lipids, which was determined by the concentration of TBARS (thiobarbituric acid reactive substances) after egg storage for 28 days at 18 °C. Lutein additive also significantly increased the vitamin A content in the yolk and reduced the amount of vitamin E.

In a later experiment, the same authors [66] used marigold flower extract (0, 150, 250, and 350 g/t of feed) as a source of carotenoids, and compared the effectiveness of this extract (at a dose of 350 g/t) and a mixture of synthetic carotenoids (Carophyll red and yellow) in a production environment. An increase in the dose of marigolds led to a significant increase in the indices of redness and yellowness of the yolk ($p < 0.001$) and a decrease in the lightness index ($p < 0.05$). In this case, synthetic carotenoids in an industrial experiment reduced ($p < 0.001$) the yellowness index compared to the control without carotenoid additives. The deposition of xanthophylls in the yolk with an increase in the dose of marigold in the diet increased ($p < 0.001$): for lutein from 18.1 (control) to 29.8 mg/kg of yolk dry matter (dose of marigold 350 g/t), for zeaxanthin − from 12.3 to 19.2 mg/kg, respectively. It should be noted that in the industrial experiment, the introduction of synthetic carotenoids into the diet led to a decrease of almost 10% ($p < 0.001$) in the vitamin E content in the egg yolk (α-tocopherol), whereas in the group receiving marigolds, it did not have significant differences with the control. The authors were unable to identify a natural or synthetic carotenoid that affected the deposition of vitamins in the egg yolk. The authors consider the optimal dose of flour from the flowers of marigold to improve the color of the yolk is 250 g/t, and in terms of the economy of egg production — since at a dose of 250 g/t, the productivity indicators of the layers are slightly reduced. A series of experiments in which synthetic and natural sources of carotenoids were compared [59] led to the same conclusions.

In another experiment, Englmaierová et al. [67] introduced marigold extract with a lutein content of 21.26 and zeaxanthin 9.65 mg/kg in a corn-wheat diet at doses of 0, 150, 350, 550, 750 and 950 g/t of feed. An increase in the laying egg rate of laying hens in the 550 and 950 g/t variants and an increase in the weigh of eggs in the groups receiving the extract in doses of 550 and 750 g/t were revealed. The indices of redness and yellowness of the yolk increased with increasing the dose of the extract, and the index of lightness decreased. The content of lutein and zeaxanthin in egg yolks increased from 12.34 and 5.92 mg/kg of dry matter (control), respectively, to 36.33 and 25.59 mg/kg (extract dose 950 g/t). The authors believe that the extract at a dose of 550 g/t can be used as a replacement for synthetic xanthophylls.

The experiment of Spada et al. [68] compared the effects of natural (South American annatto shrubs) and synthetic (a mixture of red and yellow Carophyll) carotenoid sources in laying hens' diets on egg yolk color, the organoleptic properties of fresh and stored eggs (boiled and fried), the TBARS concentration in eggs and the emulsion properties of the yolk. It was established that the score for the color of the yolk of freshly boiled eggs from a bird receiving both sources of carotenoids was significantly higher than in the control group ($p < 0.05$); this difference gradually leveled during the storage of eggs. No significant differences in scores for the smell and texture of the yolk were observed. The control received higher scores for the color of the yolk of fried eggs, which was associated with the excessive, according to tasters, redness of the yolk from hens that received carotenoids. Lipid peroxidation (which was evaluated via the concentration of TBARS) during egg storage occurred in all groups, but in the eggs from hens that received carotenoids, it progressed somewhat more slowly. The emulsion properties of the yolk, which were evaluated by the electrical conductivity of yolk emulsions of different concentrations, did not undergo significant changes when both sources of carotenoids were introduced into hens' rations. The authors conclude that a natural source can be used as a rather effective replacement for synthetic carotenoids; however, to achieve the necessary organoleptic and technological properties of the yolk, it is necessary to more carefully select the dosage of preparations, especially synthetic ones.

The presence of carotenoid esters in natural feed preparations for laying hens not only impairs the absorption and transport of carotenoids into the yolk, but can also affect the fatty acid picture of the yolk lipids. The experiment of Altunta et al. [69] considers the effect of feeding hens with flour from *Tagetes* flowers (10 or 20 g/kg feed) for 42 days on the fatty acid picture of yolk lipids of eggs laid in the last week of the experiment. In contrast to the study described above [67], here the egg weight at a dose of marigold 20 g/kg was lower than in the control. The addition of marigold increased the concentration in the yolk of the sum of saturated fatty acids and reduced the content of the sum of monounsaturated fatty acids. The authors attribute this to the fact that in marigolds, a certain part of lutein is in the form of esters with saturated palmitic and myristic acids. At the same time, such esters previously showed a very insignificant degree of deposition in the yolk, i.e. about 5% of the dose in the diet [53].

Enrichment of the diet with xanthophylls has a positive effect on the state of hens' bodies [70]. Gao et al. [71] studied the effect of introducing a mixture of xanthophylls (40% lutein and 60% zeaxanthin; 20 or 40 g/t) into the diet of laying hens at the age from the 34th week on liver and blood condition indicators characterizing the activity of the antioxidant system (activity of glutathione peroxidase, superoxide dismutase and catalase, total antioxidant capacity, the ratio of oxidized and reduced forms of glutathione, concentration of malondialdehyde). An increase in the activity of antioxidant enzymes and a decrease in the concentration of malondialdehyde have been established.

Since in eggs, especially dietary and/or functional eggs, an increase in the proportion of saturated fatty acids in yolk lipids is undesirable, the best sources of xanthophylls in laying hens' diets are synthetic products where they are not esterified with saturated fatty acids. It is also possible to use natural extracts of xanthophyll-rich plants previously hydrolyzed to saponify xanthophyll esters. Their use may be due to economic considerations, i.e. the availability and lower cost of natural sources compared to synthetic ones. At the same time, in the production of "organic", environmentally friendly eggs, it is quite possible to use intact natural sources of xanthophylls, which, if the composition and dose are selected correctly, can quite effectively replace synthetic carotenoids without significantly impairing the productivity of laying hens. A similar conclusion was made by the authors of many papers, where synthetic and natural carotenoids from terrestrial plants [59, 65-68] or seaweeds were compared [72].

When using natural sources of xanthophylls (especially saponified extracts), it is necessary to consider their instability in contact with atmospheric oxygen, as well as with certain nutrients and trace elements (for example, iron) that may be present in laying hens' diet. So, when storing an extract from marigold flowers, the content of carotenoids (in particular, β-carotene and lutein), total phenolic and total flavonoid compounds, as well as antioxidant activity, are gradually reduced, and the process speed depends on storage temperature: the most effective temperature was –20 °C, and its increase to +4 °C and further contributed to a faster decrease in indicators. The authors conclude that the best conditions for storing this product are air elimination with freezing up to –20 °C [73]. Therefore, after opening the factory packaging containing such preparation, it must be used as quickly as possible. Another method for stabilizing carotenoids, facilitating their transport through the digestive tract to the place of absorption (duodenum and jejunum) without associated oxidation, is solubilization in vegetable oils or absorption on lipid solids [74, 75].

Thus, correctly selected sources of carotenoids, primarily lutein and zeaxanthin, in laying hens' diets allow obtaining eggs with the content of these and other carotenoids 2-3 mg/egg or more. Since the requirements for consumption of carotenoids (especially those that do not have A-provitamin activity) by humans are almost absent, it is difficult to say that eggs enriched to such an extent satisfy the quantitative criterion of functionality, according to which the product in an amount corresponding to its required daily intake should provide at least 30% of the recommended daily intake of the target substance. However, this percentage of the daily requirement may be significantly higher than 5-10%, i.e. the threshold specified by earlier studies on egg enrichment with carotenoids, which many authors of the papers of the last two decades agree with.

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