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THE ROLE OF CAROTENOIDS IN THE BIOFORTIFICATION OF TABLE CHICKEN (*Gallus gallus* L.) EGGS WITH ω-3 POLYUNSATURATED FATTY ACIDS, VITAMIN E, AND SELENIUM

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Abstract

Natural fortification of plant and animal derived foodstuffs with essential nutrients (biofortification) is regarded by modern nutritional science as an effective alternative for the synthetic food additives. Biofortified eggs are usually enriched with one or two target micronutrients via layer diets. However, the nutrition of the large part of the World's population (both adult and infant) is characterized by the simultaneous deficiencies of different micronutrients: vitamins, carotenoids, minerals, and polyunsaturated fatty acids (PUFAs). The combined enrichment of foodstuffs with balanced set of the essential nutrients can compensate for these deficiencies and control the secondary deficiencies of the nutrients which can become deficient due to the changes in the diets of the patients. In the study presented the combined biofortification of table chicken eggs with four deficient micronutrients with different chemical and physiological properties was examined. The trial was performed in 2019 on four treatments of cage-housed Leghorn laying hens (*Gallus gallus* L., cross SP 789, 30 birds per treatment) during 60 days of the productive period. The concentrations of ω-6 and ω-3 PUFAs (from flaxseed oil and cake) in the diet for control treatment 1 were 2.18 and 1.97 %, respectively, ω-6/ω-3 ratio 1.11:1; the diet was also supplemented with vitamin E (150 ppm, as concentrated D-α-tocopherol) and selenium (0.5 ppm as 1:1 mixture of organic (Sel-Plex®) and inorganic (sodium selenite) forms); the background concentration of carotenoids in this diet was 7.5 ppm. Treatments 2, 3, and 4 were fed the same diet additionally supplemented with 10; 14 and 18 ppm of carotenoids (as extract of the marigold, *Tagetes erecta*), respectively. The supplementation with carotenoids of diets containing the constant combination of other three target micronutrients (that we have studied in our previous trials) did not significantly affect the egg production (45.1-46.9 eggs per hen during 60 days of the trial), feed conversion ratios (1.58-1.63 kg of feed per 10 eggs and 2.43-2.50 kg of feed per 1 kg of eggs laid), and egg weight and morphology (average egg weight 65.0-65.2 g, yolk weight 18.1-18.3 g, albumen weight 39.3-39.7 g, eggshell weight 7.0-7.1 g) in layers. The eggs simultaneously fortified with the four micronutrients can be the valuable source of lutein, selenium, PUFAs, and vitamin E in human diet; these eggs could be also used as a component of multi-ingredient functional foodstuffs. The concentration of carotenoids in eggs increased with the increase in their concentration in layer diets. The increase in the concentration of the carotenoids in yolk enhanced the intensity of yolk coloration 1.66-1.84-fold, improving the market appearance and consumer attractibility of the eggs. The concentration of the four target micronutrients (per 100 g of edible part of the eggs) in the eggs from the treatment fed the highest dietary level of carotenoids were 0.7 mg for carotenoids, 62 μg for selenium, 10 mg for vitamin E, 417 mg for ω-3 PUFAs (with ω-6/ω-3 ratio 3.1:1), 3.96; 1.62; 4.37 and 2.42 times higher, respectively, in compare to "standard" (non-fortified) eggs. The daily consumption of

one fortified egg will provide a consumer with 7.5 and 12 % of recommended daily consumption of ω-6 and ω-3 PUFAs, respectively, as well as 9; 39; 51 and 8 % of recommended daily consumption of vitamin A, vitamin E, selenium, and easily digestible carotenoids (primarily, lutein), respectively.

Keywords: biofortification, lutein, chickens, eggs, vitamin E, selenium, ω-3 PUFA

Micronutrients deficiency in diets reduces the adaptive potential of a person, increasing risks of many diseases and negatively affecting performance. A simultaneous shortage of several micronutrients, e.g. vitamins, carotenoids, minerals and a deficiency of polyunsaturated fatty acids (PUFA) are characteristic of most of the adult and child population, therefore, it is advisable to simultaneously enrich food with a complex of micronutrients [1]. In addition, complex enrichment allows undesirable consequences occurred upon biofortification with one nutrient to be avoided. For example, the excessive intake of PUFA enhances lipid peroxidation both in the fortified foodstuff itself and in a person consuming such products, as a result, the body's need for vitamin E increases [2]. Simultaneous enrichment with PUFA and α-tocopherol or carotenoids with antioxidant properties (tomato paste) solves this problem [3, 4]. To enrich eggs with selenium, both organic and inorganic forms of this microelement are used [5, 6]. Flaxseed [7], marine microalgae [8], and fish oil [9] are the sources of PUFA.

Recommendations to limit the eggs eaten per day, as they are supposedly rich in cholesterol are no longer so strict. In particular, it was found that low (less than 2 eggs per week), moderate and even high egg consumption (4-7 eggs per week) is not associated with an increased risk of cardiovascular diseases in patients with or without diabetes, and with the development of metabolic syndrome [10-13], although position of some is more cautious [14]. Two eggs for 3 weeks eaten by healthy young people for breakfast did not adversely affect biomarkers of the risk of cardiovascular diseases, i.e. the ratio of low to high density lipoproteins, the blood levels of glucose and triglycerides. This created a subjectively stronger satiety for a longer period during the day compared to the persons who ate fast carbohydrates (oatmeal) for breakfast [15]. Consequently, hen eggs the value of which can be increased by enriching hens' feed with micronutrients (biofortification) become an even more attractive for these purposes. An important advantage of biofortification is that chickens are capable of a bio-transformation of enriching additives (vitamins, minerals) into their natural forms.

The main sources of lutein in human nutrition are colored vegetables and fruits, as well as chicken egg yolk, from which lutein is absorbed much better than from purified lutein or lutein from plant sources [12]. In the human body, lutein is in the eye macula and in brain [16]. Lutein is the main dietary carotenoid that prevents macular degeneration during aging, improves cognitive functions, cardiovascular health, reduces the risk of cancer [17, 18] and has anti-inflammatory effects [19]. To enrich poultry products with carotenoids, they often use either dietary natural powders from dried tomatoes [20] and/or red pepper [21], herbal additives, for example, calendula flower extract [4], microalgae spirulina [22], or plant-derived carotene-containing preparations [12, 23, 24].

The available scholar papers show that in biofortification, chicken feed is mainly enriched with one [8, 9, 20, 24] or two micronutrients [4, 5]. Our previous studies have shown the effectiveness of complex enrichment of edible eggs with three nutrients, the ω-3 PUFA, vitamin E, and selenium [10]. This communication is the first to present data on the simultaneous enrichment of chicken eggs with four deficient micronutrients, which, moreover, have a different chemical nature and differ in physiological properties. We did not find such examples in the available publications.

The work aimed to simultaneously biofortify edible eggs with carotenoids, ω-3 polyunsaturated fatty acids, vitamin E and selenium by enriching the mixed feed of laying hens and to evaluate the effect of such additives on the productive performance of birds as an indicator characterizing their general physiological status and the proposed biotechnology efficiency.

Materials and methods. The cross SP 789 hens (*Gallus gallus domesticus* L.) of the productive flock aged 300 days (vivarium of the Selection and Genetic Center Zagorskoe Experimental Breeding Farm VNITIP, Moscow Province, Sergiev Posad, 2019) were assigned to four treatments, $n = 30$ each. For all treatments the basal diet (BD) was used contained (per 1 ton of mixed feed) 57.61% wheat, 9.82% extruded semi-defatted soybeans, 12.12% sunflower meal, 5.0% flaxseed cake, 3.0% flaxseed oil, and 1.5% fatty acids; the ω-6 and ω-3 PUFA content was 2.18 and 1.97%, respectively, at the ratio of 1.11:1; the concentration of vitamin E was 150 g, of selenium 0.5 g. Dietary selenium-containing yeast Sel-Plex® (Alltech, USA), with selenium in the form of selenomethionine (50%) and selenocystine (25%), and sodium selenite were used at the ratio of 1:1. Fatty acids, the processed wastes of the fat and oil industry (OOO AVK-CHEM, Russia) which was a source of vitamin E, contained 90% fats, at least 11.3 mg/g α-tocopherol, and 280 μg/g natural carotenoids. The diets for all treatments were supplemented with 100 g/t Fidbest W (xylanase and β-glucanase preparation) and 100 g/t Fidbest R (3-phytase preparation) (OOO Sibbiopharm, Russia). In the control (group I), the BD was not supplemented with carotenoids (their natural level was 7.5 g/t feed). For three treatments, the BD was supplemented with 500, 700 and 900 g of marigold flower extract (*Tagetes erecta*) (Biofon yellow, Biokol Agro, Russia; 20 g/kg, 85% lutein and 15% zeaxanthin) as a source of carotenoids ensuring 10, 14 and 18 g of carotenoids per 1 ton of mixed feed in test groups II, III, and IV, respectively.

Poultry up to 360 days of age (from June 19 to August 18, 2019) were kept in cage batteries (FACCO, Italy) 3 birds in a cage with cage floor area of 450 cm^2 per bird.

The measured indicators were 1) the poultry viability as total percentage of live birds, 2) a bird live weight quantified by individual weighing of the entire flock at 300 and 360 days of age; 3) egg production per initial and average laying hen as calculated from the daily number of laid eggs per group; 4) the weight of eggs per group estimated by individually weighing of all eggs laid for 3 days in a row after 30 and 60 days from the beginning of the experiment; 5) the egg mass yield per initial and average layer calculated from the number of laid eggs and the average weight of one egg in each group; 6) daily feed consumption calculated as the fed feed minus feed residues at the end of each week; 7) feed costs per 10 eggs and per 1 kg egg mass calculated based on the feed consumption, egg production and egg mass yield; 8) the weight of the albumen, yolk, and eggshell assessed by separate individual weighing of the constituent parts of the egg 30 and 60 days after the beginning of the experiment; 9) the albumen:yolk ratio calculated from the weight of egg albumen and yolk; 10) the intensity of the color of the egg yolk assessed individually according to the BASF color scale 30 and 60 days after the beginning of the experiment. The concentrations of carotenoids, vitamins A, E and B_2, ω-3 and ω-6 PUFAs were determined in the yolk, vitamin B_2 in albumen, and selenium in melange.

When determining the sum of carotenoids and fat-soluble vitamins in the egg yolk, a unified sample preparation was applied, including saponifying the samples with a 50% potassium hydroxide solution followed by extraction with diethyl ether according to Biological control during incubation of poultry

eggs: methodological instructions (Sergiev Posad, 2014). The mass fraction of A and E vitamins was determined by normal-phase high performance liquid chromatography (chromatographic system Knauer advanced scientific instruments, Knauer Engineering GmbH Industrieanlagen & Co., Germany) in accordance with the P 4.1.1672-03 Guideline for quality control and safety of biologically active food additives (Moscow, 2003). The total amount of carotenoids was measured colorimetrically (photometer KFK-3-01, ZOMZ, Russia) with potassium dichromate to construct a calibration curve at OD_{450} (blue filter). The wavelengths of 292 and 450 nm can be used for the quantitation of vitamin A and carotene, since in this region their absorption spectra practically do not overlap [25]. The components were separated in a Luna 5 μm Silica(2) 100 A New Column 150×4.6 mm (Phenomenex, United States), eluted with a hexane:isopropyl alcohol mixture (98:2). Vitamins A and E concentrations were estimated at 292 and 324 nm, with Retinol Sigma cat. No. R 7632 (Sigma-Aldrich, USA) and (+/–)-α-Tocopherol Fluka cat. No. 95240 (Fluka, Germany) as standards.

Water-soluble vitamin B_2 (riboflavin) in the egg yolk and albumen was determined fluorographically using a Fluorat-02-3M liquid analyzer (NPFNP Lumex, RF). Sample preparation included alcohol extraction (from albumen with 96% ethanol, from yolk with 55% ethanol) followed by filtration through a medium-pore paper filter ("yellow strip"). The intensity of fluorescence in ultraviolet rays was measured, the concentration of riboflavin was calculated vs. a standard solution of vitamin B_2.

The selenium concentration in the melange was determined by atomic absorption spectrometry with electrothermal atomization (a Duo 240 FS/240Z spectrometer, Varian, USA). The samples were decomposed using a Milestone START D microwave sample preparation system (Milestone Systems, Italy) with 1% nickel nitrate $Ni(NO_3)_2$ solution as a modifier. The calibration graph was constructed based on the dilutions of a standard sample Se (IV) GSO No. 7779-2000 (1 mg/cm^3, EAA Ecoanalytika, Russia).

The mass fraction of crude fat was measured by the Randall method using an extractor VELP Ser148 (VELP, Italy), the fatty acid composition was determined by capillary gas-liquid chromatography (a Kristall-2000M gas chromatograph, ZAO SBK Khromatek, Russia). The extracted lipids were transesterified by an acid catalyst (hydrogen chloride) in the presence of an excess of methyl alcohol. Methyl esters of fatty acids were separated (a Stabilwax®-DA capillary column, Restek, United States; length 60 m, inner diameter 0.32 mm, phase thickness 0.5 μm) and recorded (a Kristall 2000M flame ionization detector, CJSC SBK Khromatek, Russia). The CRM47885 kit (Sigma-Aldrich, USA) served as a standard for fatty acids. The mass fraction of an individual fatty acid from the total fatty acids was calculated by the internal normalization method.

The data were processed by the methods of variation statistics with Microsoft Excel software. The tables and the figure show the means (M) and their standard errors (±SEM). The statistical significance of differences between groups was assessed by Student's t-test at $p < 0.05$.

Results. The test showed a 100% viability in all groups over a 60-day period (Table 1). We did not note significant differences between the groups in the hen live weight at 360 days of age and the average weight of eggs over the experiment. According to Czech scientists [26], the addition of lutein extract (90%) to the diet of hens at a dose of 250 mg/kg significantly increased the weight of eggs, the thickness and strength of the shell.

1. Productivity performance of cross SP 789 laying hens (*Gallus gallus domesticus* L.) fed diets enriched with biofortification nutrients (*M*±SEM, vivarium of the Selection and Genetic Center Zagorskoe Experimental Breeding Farm VNITIP, Moscow Province, Sergiev Posad, 2019)

Parameter	Group			
	I (control, $n = 30$)	II ($n = 30$)	III ($n = 30$)	IV ($n = 30$)
Viability, %	100	100	100	100
Live weight, g:				
300 days	1579±24	1577±23	1579±13	1574±24
360 days	1650±33	1722±29	1701±28	1674±23
Eggs per initial and average hen	45,1±4,2	45,8±3,8	46,3±5,1	46,9±4,9
Egg laying intensity, %	75,2±6,8	76,3±5,3	77,2±6,6	78,2±7,1
Average egg weight, g	65,0±0,5	65,1±0,6	65,2±0,4	65,1±0,4
Egg mass outcome per hen, kg	2,93±0,32	2,98±0,27	3,02±0,45	3,05±0,38
Feed consumption:				
per head/day, g	122,2±11,3	122,2±12,2	122,8±10,9	123,5±11,5
per 10 eggs, kg	1,63±0,12	1,60±0,11	1,59±0,09	1,58±0,11
per 1 kg of egg mass laid, kg	2,50±0,31	2,46±0,28	2,44±0,26	2,43±0,34

N o t e. For diets supplemented with carotenoids, ω-3 polyunsaturated fatty acids, vitamin E, and Se according to the treatments, see *Materials and methods.*

Group IV surpassed the other groups in the egg yield per hen by 1.3-4.0% and in egg mass yield per hen by 1.2-4.3%. Note that these indicators were minimal in control (group I). It was reported [27, 28] that the inclusion of calendula flower extract or corn gluten as a dietary source of carotenoids provided a 7-14% increase in egg production of laying hens. According to other report [29), neither the type of lutein source (flour from marigold flowers and a hydrolyzed extract of this flour in which lutein esters were saponified), nor its dose in the diet (10, 20, 30 and 40 g t) did not significantly affect egg productivity and the main markers of egg quality.

Feed intake per hen per day was the lowest in the control and in group II, with a 0.65-1.05% decline compared to groups III and IV, and maximal in group IV. In addition, higher egg production and egg mass yield in this group resulted in a decrease in feed consumption by 0.63-3.07% per 10 eggs and by 0.41-2.80% per 1 kg of egg mass laid compared to other groups. Feed consumption was the highest in the control.

Table 2 shows that, over a 60-day experiment, the groups did not differ reliably in the weight (absolute and relative) of yolk, albumen and eggshell, calcium content in the eggshell, the contents of vitamins A, E, B_2 in yolk, the content of B_2 in the albumen, and in the albumen-to-yolk proportion. The concentrations of selenium, ω-3 PUFA and the ratio ω-6/ω-3 between the groups differed insignificantly. The observed tendency towards an increase in the vitamin E level in the yolk with an increase in the dose of dietary carotenoids, though does not reach statistical significance, may indicate their certain synergy.

2. Egg morphological and biochemical indicators of cross SP 789 laying hens (*Gallus gallus domesticus* L.) fed diets enriched with biofortification nutrients (*M*±SEM, vivarium of the Selection and Genetic Center Zagorskoe Experimental Breeding Farm VNITIP, Moscow Province, Sergiev Posad, 2019)

Показатель	Group			
	I (control, $n = 30$)	II ($n = 30$)	III ($n = 30$)	IV ($n = 30$)
Weight:				
yolk, g	18.1±0.2	18.2±0.2	18.2±0.2	18.3±0.1
yolk, %	28.0	28.2	28.0	28.3
albumen, g	39.5±0.3	39.3±0.4	39.7±0.3	39.3±0.2
albumen, %	61.0	60.9	61.2	60.8
eggshell, g	7.1±0.1	7.0±0.1	7.0±0.1	7.0±0.2
eggshell, %	11.0	10.9	10.8	10.9

Continued Table 2

Intensity of yolk color, points	3.8±0.1	6.3±0.2*	6.7±0.2*	7.0±0.3*
Albumen to yolk ratio	2.2	2.2	2.2	2.1
Concentration:				
calcium in eggshell, %	36.6±3.3	36.6±2.4	36.1±3.8	36.4±2.9
in yolk, μg/g:				
carotenoids	5.6±1.1	13.0±2.6*	14.4±2.9*	21.9±4.4*
vitamin A	4.2±0.3	4.1±0.4	4.5±0.2	4.6±0.5
vitamin E	283±21	290±17	306±23	315±29
vitamin B_2	5.6±0.4	6.5±0.7	5.8±0.2	5.8±0.4
vitamin B_2 in albumen, μg/g	3.9±0.4	4.3±0.3	4.3±0.6	4.3±0.4
Concentration per 100 g edible part of the egg:				
carotenoids, μg	177±35	424±85	454±91	701±140
selenium, μg	62±0.7	61±0.7	62±0.9	62±1.1
vitamin E, mg	8.9±0.9	9.5±1.2	9.6±0.8	10.1±1.4
ω-6 PUFA, mg	1093±52	1075±75	1140±67	1298±111
ω-3 PUFA, mg	347±19	393±31	412±43	417±28
ω-6 PUFA/ω-3 PUFA	3.1:1	2.7:1	2.8:1	3.1:1

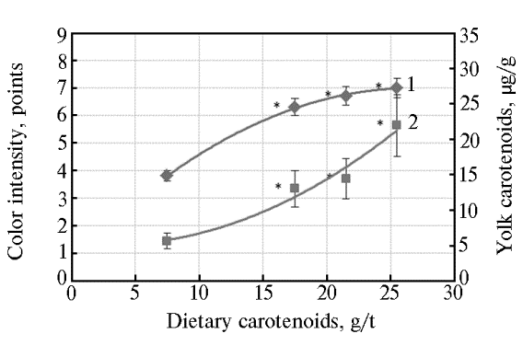
N o t e. PUFA — polyunsaturated fatty acids. For diets supplemented with carotenoids, ω-3 polyunsaturated fatty acids, vitamin E, and Se according to the treatments, see *Materials and methods.*

* Differences between treatment groups and control are statistically significant at $p < 0.05$.

Our findings do not agree with other investigations (26) which noted a significant increase in the yolk vitamin A level and a decrease in the vitamin E level in layers fed dietary extract of lutein powder (250 g/t), which may be due to a smaller amount of the dietary extract (10-18 g/t) in our trials. We also note that in a later work by the same researchers [30], when the marigold flower extract was used, the vitamin E concentration remained at the control level.

The biochemical indicators of eggs in the best group IV are noteworthy, e.g. 0.7 mg of carotenoids per 100 g edible egg part, 62 μg selenium, 10 mg vitamin E, 417 mg ω-3 PUFA with the ω-6/ω-3 ratio of 3.1:1. So the increase is 3.96-fold ($p < 0.05$), 1.62-fold ($p < 0.001$), 4.37-fold ($p < 0.01$), and 2.42-fold ($p < 0.001$), respectively, as compared to unfortified eggs [10].

We assessed the color intensity and quantified carotenoids in the yolk as dependent on the fed diet (Fig.). The saturation curve for the first parameter gradually rose to a maximum, which was 3.9 times higher than in the control group ($p < 0.05$). Similarly, with an increase in the dose of dietary carotenoids, their concentration in the yolk significantly increased. A dose of carotenoids of about 20 g/t feed ensured their maximum concentration in the yolk. The poultry feed enrichment with carotenoids was accompanied by an increase in their amount in the yolk to values typical for eggs of poultry fed diets with an increased level of these micronutrients [29, 31, 32].



Egg yolk color intensity (1) **and the concentration of carotenoids** (2) **depending on the dose of dietary carotenoids fed to cross SP 789 laying hens (*Gallus gallus domesticus* L.) for biofortification.** The points correspond to groups I-IV (from left to right) according to the level of dietary carotenoids (see *Materials and methods*). An asterisk (*) marks values that statistically significantly ($p < 0.05$) differ from the control (*M*±SEM, vivarium of the Selection and Genetic Center Zagorskoe Experimental Breeding Farm VNITIP, Moscow Province, Sergiev Posad, 2019).

We evaluated the egg indicators reached upon biofortification as a percentage of the recommended [33] or adequate level of a nutrient consumption [34] for humans (Table 3). Due to complex biofortification, one egg per day will provide 7.5% ω-6 PUFA, 12.0% ω-3 PUFA (at a 3.1:1 proportion), 39% vitamin

E and 51% Se of their recommended or adequate consumption. Thus, the resulting eggs meet the criteria for micronutrient-fortified food. As for carotenoids, their level increased 4 times, reaching 8% of the adequate daily intake. E.R. Kelly et al. [35] found that the increase in blood lutein levels in volunteers due to 0.9 mg of lutein in eggs was the same as for 5 mg of this carotenoid from a dietary supplement. Given that lutein from egg yolk is absorbed much better than from plant sources [12], we can assume that the nutritional value of the product we suggested has increased significantly.

3. Micronutrient value of eggs of cross SP 789 laying hens (*Gallus gallus domesticus* L.) fed dietary carotenoids for biofortification (*M*±SEM, vivarium of the Selection and Genetic Center Zagorskoe Experimental Breeding Farm VNITIP, Moscow Province, Sergiev Posad, 2019)

Micronutrient	Recommended consumption (adequate consumption)	Concentration per fortified egg (group IV)	Compensation of daily reguirements, %
Carotenoids	(5 mg)	0.4 mg	8
Selenium	55-70 μg	35.7 μg	51
Vitamin E	15 mg	5.8 mg	39
Vitamin A	900 мμg	84 μg	9
ω-6 PUFA	(10 g)	0.748 g	7.5
ω-3 PUFA	(2 g)	0.240 g	12

Note. PUFA — polyunsaturated fatty acids. For diets supplemented with carotenoids, ω-3 polyunsaturated fatty acids, vitamin E, and Se according to the treatments, see *Materials and methods*.

However, remember that the consumption of two eggs, on the one hand, will overcome a 15% recommended intake for carotenoids and vitamin A, which is the lower limit for classification of the eggs as an enriched product. On the other hand, the consumption of selenium, although in a less dangerous organic form, will reach 100% of the physiological requirement. Selenium is also found in other food products. Therefore, it seems advisable to somewhat decline Se level in the diet of laying hens given that the upper permissible consumption limit for an adult person is 150 μg per day [33].

Yolks with a maximum level of selenium, PUFA, vitamin E and lutein will increase the micronutrient value of this component, not only when eaten directly. Eggs enriched with micronutrients can be a good raw material for producing melange, a component of functional food products. Experiments on animals have shown that carotenoids are well absorbed from the enriched eggs [36]. High preservation of carotenoids in an omelet of eggs enriched by feeding chickens with corn grain rich in carotenoids (about 85% of carotenoids in a raw egg) [37] shows that heat treated eggs can serve as a source of these micronutrients in the diet of the population. PUFAs are also still stable in boiled eggs [38]. Consumption of eggs enriched with PUFA (6 pcs per week) for 8 weeks led to an increase in the amount of docosahexaenoic acid in the membranes of erythrocytes in healthy volunteers [39]. The use of whole boiled eggs has proven to be effective to increase the absorption of α- and γ-tocopherol from fresh salad in healthy young adults [40]. The property of the phospholipids of chicken egg yolk to act as a "vehicle" for carotenoids was used to create a component based on yolks, pumpkin, and carrots for enriching curd products and dairy drinks with carotenoids [41].

So, our work has led us to conclude that the simultaneous supplementation with dietary carotenoids, ω-3 polyunsaturated fatty acids (PUFA), selenium and vitamin E provides a significant increase in their levels in chicken eggs. Due to higher level of carotenoids in the yolk, its color becomes more intense, which improves consumer qualities. Dietary carotenoids, upon constant profiles of other three micronutrients tested previously, did not significantly affect productivity

performance of hens, with 45.1-46.9 eggs over 60 days, feed conversion rate (1.58-1.63 kg per 10 eggs and 2.43-2.50 kg per 1 kg egg mass), and the egg, yolk, albumen and eggshell weight (65.0-65.2 g, 18.1-18.3 g, 39.3-39.7 g, and 7.0-7.1 g, respectively). However, there was a 2.40-3.96-fold increase in the concentration of carotenoids. In the best group, the concentration of carotenoids reached 0.7 mg per 100 g of edible part, selenium reached 62 μg, vitamin E reached 10 mg, and ω-3 PUFA reached 417 mg (at 3.1:1 proportion of ω-6/ω-3). These are 3.96 times higher ($p < 0.05$), 1.62 times higher ($p < 0.001$), 4.37 times higher ($p < 0.01$), and 2.42 times higher ($p < 0.001$), respectively, then in unfortified eggs. One egg per day will provide a person with additional intake of 7.5 and 12% ω-3 and ω-6 PUFAs, 9% of vitamin A, 39% of vitamin E, 51% of selenium and 8% of easily assimilated carotenoids (mainly lutein) regarding the recommended doses. Eggs enriched with a complex of four micronutrients are both valuable natural food product, providing with lutein, selenium, PUFA and vitamin E, and an ingredient of other functional foodstuffs, which facilitates assimilation of other dietary components.

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