The efficiency of xanthophylls in the combined fortification of table eggs

A Sh Kavtarashvili, E N Novotorov and I L Stefanova

Federal Scientific Center “All-Russian Research and Technological Poultry Institute” of Russian Academy of Sciences, 10, Ptitsegradskaya Str., Sergiev Posad, Moscow Province, 141311, Russia

E-mail: alexk@vnitip.ru

Abstract. The efficiency of xanthophylls in the combined enrichment of table chicken eggs with ω-3 polyunsaturated fatty acids (PUFAs), vitamin E (VE), selenium (Se), and carotenoids via supplementation of diets for laying hens was studied on 4 groups of cage-housed SP-789 layers (30 birds per group). All groups received a diet containing 2.18% of ω-6 and 1.97% of ω-3 PUFAs, 150 ppm of VE, 0.5 ppm of Se, and 7.5 ppm of total carotenoids; diets for groups 2-4 were additionally supplemented with xanthophylls (10, 14, and 18 ppm of marigold extract “Biofon Yellow”). It was found that the concentrations of ω-6 and ω-3 PUFAs per 100 g of edible parts of the eggs from groups 1-4 were 1093 and 347, 1075 and 393, 1140 and 412, 1298 and 417 mg, respectively. Se amounted to 61.8, 60.9, 62.1, 61.9 μg. Vitamin E was 8.9, 9.51, 9.63, 10.1 mg. Total carotenoids amounted to 176.6; 424.4; 454.2; 701.4 μg. The intensiveness of yolk coloration was 3.8; 6.3; 6.7; 7.0 scores, respectively. The best results were found in group 4.

1. Introduction

In recent decades functional foodstuffs with beneficial effects on the consumers’ health increasingly have drawn the attention of food scientists. These foodstuffs can be enriched with different bioactive substances (BAS): vitamins, trace elements, ω-3 polyunsaturated fatty acids (PUFAs), antioxidants, pro- and prebiotics, etc. [1-4]. Eggs and poultry meat (which per se are the most cheap and available sources of valuable protein and essential fatty acids) can be functional foodstuffs due to the higher efficiency of the presence of different nutrients and BAS in the diets of poultry in comparison to other diets of food-producing animals [5]. It is known that enrichment of foodstuffs with PUFAs enhances the lipid peroxidation within the foodstuffs during the storage and cooking. This problem could be solved via the concurrent enrichment of these foodstuffs with antioxidants [6] stabilizing the lipids within the foodstuffs, which is beneficial for the consumers’ health. An optimal ω-6/ω-3 PUFAs ratio in human diet should be 2-3:1 [7].

Xanthophylls which earlier served for the improvement of egg yolk coloration and consumer attractiveness of the eggs are presently also regarded as target BAS in the production of functional eggs [8, 9]. Xanthophylls (primarily lutein and zeaxanthin) are the exclusive carotenoids presented in the retina and lens and protecting the eye [10]. These xanthophylls protect photoreceptors and retinal epithelium from the harmful effects of the blue light [11] and decrease the light-induced oxidative stress in the eye tissues [12].
Our earlier research evidenced the reasonability of concurrent enrichment of chicken eggs with ω-3 PUFAs, vitamin E (VE), and selenium (Se); the optimal levels of inclusion of these BAS into the diets for laying hens were determined [13]. However, the data on the concurrent enrichment of eggs with four bioactive micronutrients still cannot be found in the literature.

2. The objective of the study
The objective of the present study was the investigation of the efficiency of xanthophylls in the combined enrichment of table chicken eggs with ω-3 PUFAs, VE, Se, and carotenoids via supplementation of diets for laying hens.

3. Material and methods
The trial was performed on 4 groups of commercial layers (cross SP-789) from 300 to 360 days of age (30 cage-housed birds per group, 3 hens per cage). All groups received the wheat-based diet with balanced concentrations of macro- and micronutrients (wheat - 57.61%, extruded semi-defatted soybean - 9.82%, sunflower cake - 12.12%, flaxseed cake - 5.00%, flaxseed oil - 3.00%, enzyme preparations Feedbest W (0.01%) and Feedbest P (0.01%), preparation “Fatty Acids” - 1.50%, Sel-Plex - 500 ppm, sodium selenite - 0.55 ppm). Dietary concentrations of ω-6 and ω-3 PUFAs were 2.18 and 1.97%, respectively, with their ratio of 1.1:1; a concentration of VE (D-α-tocopherol) of 150 ppm; concentration of Se (with equal proportion of organic and inorganic Se) of 0.5 ppm.

Diets for experimental groups 2, 3, and 4 were additionally supplemented with marigold extract (“Biofon Yellow”, containing 20 g/kg of xanthophylls, 85% of lutein and 15% of zeaxanthin) in doses of 500, 700, and 900 ppm, or 10, 14, and 18 ppm of xanthophylls, respectively. The diet for control group 1 was not supplemented with xanthophylls; the background concentration of carotenoids in this diet was 7.5 ppm.

The important parameters were mortality, live bodyweight, egg production (throughout the entire period of trial), egg weight, output of egg mass per hen, feed consumption, feed conversion ratios (FCR, as feed expenses (kg) per 10 eggs and per 1 kg of eggs laid), weights of albumen, yolk, and eggshell, albumen/yolk ratio, the intensity of yolk coloration (using BASF color scale). The concentrations of carotenoids, vitamins A, E, and B2, ω-3 and ω-6 PUFAs in yolk, vitamin B2 in albumen, Se in melange were determined. Concentrations of vitamins A and E were determined by HPLC on the Knauer system (Knauer Engineering GmbH. Industrieanlagen & Co., Germany). The total carotenoids by colorimetry were checked on photometer KFK-3-01 (ZOMZ, Russia). Vitamin B2 (riboflavin) in yolk and albumen was studied by fluorometry on liquid fluorometer Florat-02-3M (Lumex, Russia). Se in melange was analyzed by atomic absorption spectrometry with electrothermal atomization on spectrometer Duo 240 FS/240Z (Varian, USA). The concentration of crude fat was determined using Randall’s method and extractor “VELP Ser148”; fatty acid profiles were determined by capillary GLC on gas chromatograph “Crystall-2000M” (Chromatech, Russia) using reagent kit AA S18 (Sigma-Aldrich, USA).

4. Results and discussion
There were no mortality cases recorded throughout the 60-day trial period. There were no significant differences between the groups in live bodyweight in hens at 360 days of age (1.65; 1.72; 1.70 and 1.67 kg with regard to groups 1-4) and average egg weight (65.0; 65.1; 65.2 and 65.1 g).

The best egg production and egg mass output per hen were recorded in group 4 (46.9 eggs and 3.05 kg, respectively), which was higher by 1.3-4.0 and 1.2-4.3% in comparison to groups 1 (45.1 eggs and 2.93 kg), 2 (45.8 eggs and 2.98 kg), and 3 (46.3 eggs and 3.02 kg).

The lowest feed consumption was in groups 1 and 2 (122.2 g/hen/day), lower by 0.65 and 1.05% in comparison to group 3 (122.8 g/hen/day) and 4 (123.5 g/hen/day), respectively. However, the lowest FCRs were found in group 4 (1.58 kg per 10 eggs and 2.43 kg per 1 kg of eggs laid), which was lower by 3.07 and 1.25%, respectively, in comparison to group 1 (1.63 and 2.46 kg). That was lower by 0.63
and 2.80% in comparison to group 2 (1.60 and 2.46 kg), and by 1.22 and 0.41% - in comparison to groups 3, 4 (1.59 and 2.44 kg).

The data on egg morphology (Table 1) evidenced that at 330 and 360 days of age there were no significant differences between the groups in absolute and relative weights of albumen, yolk, and eggshell, as well as in the albumen/yolk ratio.

The intensity of yolk coloration increased with age of hens and with the increase in dietary concentration of xanthophylls. The highest average color intensity was found in group 4, which was 1.8-fold higher in comparison to control where this parameter was the lowest.

### Table 1. The morphology of eggs

| Parameter                         | Group |
|-----------------------------------|-------|
|                                   | 1(с) | 2   | 3   | 4    |
| **At 330 days of age**            |      |     |     |      |
| albumen, g                        | 39.1±0.50 | 39.4±0.57 | 39.5±0.32 | 39.2±0.30 |
| %                                 | 60.8  | 60.9 | 61.0 | 60.8 |
| yolk, g                           | 18.4±0.21 | 18.4±0.34 | 18.4±0.27 | 18.4±0.22 |
| %                                 | 28.6  | 28.4 | 28.4 | 28.6 |
| eggshell, g                       | 6.8±0.16 | 6.9±0.18 | 6.9±0.10 | 6.8±0.17 |
| %                                 | 10.6  | 10.7 | 10.6 | 10.6 |
| Intensity of yolk coloration, BASF scores | 3.7±0.16 | 5.5±0.22 | 5.7±0.15 | 5.7±0.19 |
| Albumen/yolk ratio                | 2.1   | 2.1  | 2.2  | 2.1  |
| **At 360 days of age**            |      |     |     |      |
| albumen, g                        | 39.9±0.33 | 39.2±0.45 | 39.8±0.42 | 39.4±0.30 |
| %                                 | 61.3  | 61.0 | 61.2 | 60.8 |
| yolk, g                           | 17.8±0.33 | 18.1±0.36 | 18.2±0.24 | 18.2±0.19 |
| %                                 | 27.3  | 28.1 | 28.0 | 28.1 |
| eggshell, g                       | 7.4±0.12 | 7.0±0.10 | 7.0±0.11 | 7.2±0.21 |
| %                                 | 11.4  | 10.9 | 10.8 | 11.1 |
| Intensity of yolk coloration, BASF scores | 3.9±0.13 | 7.1±0.17 | 7.7±0.13 | 8.4±0.13 |
| Albumen/yolk ratio                | 2.2   | 2.2  | 2.2  | 2.1  |
| **In average**                    |      |     |     |      |
| albumen, g                        | 39.5±0.30 | 39.3±0.36 | 39.7±0.27 | 39.3±0.21 |
| %                                 | 61.0  | 60.9 | 61.2 | 60.8 |
| yolk, g                           | 18.1±0.20 | 18.2±0.24 | 18.2±0.18 | 18.3±0.14 |
| %                                 | 28.0  | 28.2 | 28.0 | 28.3 |
| eggshell, g                       | 7.1±0.11 | 7.0±0.11 | 7.0±0.07 | 7.0±0.28 |
| %                                 | 11.0  | 10.9 | 10.8 | 10.8 |
| Intensity of yolk coloration, BASF scores | 3.8±0.10 | 6.3±0.20 | 6.7±0.20 | 7.0±0.28 |
| Albumen/yolk ratio                | 2.2   | 2.2  | 2.2  | 2.1  |

The chemical composition of eggs is presented in Table 2. The concentrations of calcium in the eggshell; vitamins A, E, and B2 in yolk; vitamin B2 in albumen at 330 and 360 days of hens’ age were similar. The concentration of total carotenoids in yolk increased with the increase in dietary concentration of xanthophylls in groups 2, 3, and 4; maximal concentration was found in group 4, 1.5-3.9-fold higher in comparison to other groups, while minimal concentration was found in control group 1.

The trend was found in higher VE concentration in yolk with the increase in dietary concentration of xanthophylls evidencing certain synergism between these antioxidants.
Table 2. Chemical composition of eggs

| Parameter                                | Group |
|------------------------------------------|-------|
|                                          | 1(c)  | 2   | 3   | 4   |
| At 330 days of age                       |       |     |     |     |
| Concentrations:                          |       |     |     |     |
| in eggshell, %: Ca                       | 36.60 | 36.64| 36.05| 36.78|
| in yolk, µg/g:                           |       |     |     |     |
| total carotenoids                        | 5.55  | 13.35| 14.15| 22.46|
| vitamin A                                | 4.12  | 4.23 | 3.02 | 4.33 |
| vitamin E                                | 278.3 | 285.8| 298.4| 305.8|
| vitamin B2                               | 5.56  | 6.61 | 5.52 | 6.00 |
| in albumen, µg/g: vitamin B2             | 4.10  | 4.80 | 4.18 | 4.12 |
| Concentration in 100 g of edible egg parts: |       |     |     |     |
| carotenoids, µg                          | 177.6 | 424.5| 450.0| 716.5|
| Se, µg                                   | 61.2  | 60.8 | 61.7 | 61.3 |
| vitamin E, mg                            | 8.91  | 9.09 | 9.49 | 9.76 |
| ω-6 PUFAs, mg                            | 1084  | 1069 | 1134 | 1285 |
| ω-3 PUFAs, mg                            | 341   | 388  | 406  | 412  |
| ω-6/ω-3 ratio                            | 3.2:1 | 2.8:1| 2.8:1| 3.1:1|
| At 360 days of age                       |       |     |     |     |
| Concentrations:                          |       |     |     |     |
| in eggshell, %: Ca                       | 36.61 | 36.63| 36.09| 35.98|
| in yolk, µg/g:                           |       |     |     |     |
| total carotenoids                        | 5.69  | 12.56| 14.75| 21.25|
| vitamin A                                | 4.33  | 3.93 | 4.07 | 4.90 |
| vitamin E                                | 288.2 | 294.9| 314.6| 324.4|
| vitamin B2                               | 5.70  | 6.45 | 6.12 | 5.62 |
| in albumen, µg/g: vitamin B2             | 3.74  | 3.77 | 4.36 | 4.49 |
| Concentration in 100 g of edible egg parts: |       |     |     |     |
| carotenoids, µg                          | 175.3 | 396.9| 463.2| 671.5|
| Se, µg                                   | 62.4  | 61.1 | 62.5 | 62.5 |
| vitamin E, mg                            | 8.88  | 9.32 | 9.88 | 10.25|
| ω-6 PUFAs, mg                            | 1102  | 1081 | 1146 | 1311 |
| ω-3 PUFAs, mg                            | 353   | 398  | 418  | 422  |
| ω-6/ω-3 ratio                            | 3.1:1 | 2.7:1| 2.7:1| 3.1:1|
| On average                               |       |     |     |     |
| Concentrations:                          |       |     |     |     |
| in eggshell, %: Ca                       | 36.61 | 36.64| 36.07| 36.38|
| in yolk, µg/g:                           |       |     |     |     |
| total carotenoids                        | 5.62  | 12.96| 14.45| 21.86|
| vitamin A                                | 4.23  | 4.08 | 4.55 | 4.62 |
| vitamin E                                | 283.2 | 290.4| 306.5| 315.1|
| vitamin B2                               | 5.63  | 6.53 | 5.82 | 5.81 |
| in albumen, µg/g: vitamin B2             | 3.92  | 4.29 | 4.27 | 4.31 |
| Concentration in 100 g of edible egg parts: |       |     |     |     |
| carotenoids, µg                          | 176.6 | 424.4| 454.2| 701.4|
| Se, µg                                   | 61.8  | 60.9 | 62.1 | 61.9 |
| vitamin E, mg                            | 8.90  | 9.51 | 9.63 | 10.1 |
| ω-6 PUFAs, mg                            | 1093  | 1075 | 1140 | 1298 |
| ω-3 PUFAs, mg                            | 347   | 393  | 412  | 417  |
| ω-6/ω-3 ratio                            | 3.1:1 | 2.7:1| 2.8:1| 3.1:1|

There were no significant differences between the ages (330 vs. 360 days of age) in the concentrations of ω-6 and ω-3 PUFAs, total carotenoids, VE, and Se in 100 g of edible parts of the eggs.
Eggs from groups 2, 3, and 4 had higher average concentrations of total carotenoids (2.4-4.0 times higher in comparison to control group 1) and VE (by 6.9-13.5%); the best results were found in group 4. There were no significant differences between the groups in concentrations of Se, ω-3 PUFAs, and a ω-6/ω-3 ratio.

5. Conclusions
The results of the trial evidenced the possibility of complex enrichment of table chicken eggs with ω-3 PUFAs, VE, Se, and xanthophylls by supplementation of the hens’ diets.

The experimental groups which received wheat-based diet (containing 2.18 of ω-6 and 1.97% of ω-3 PUFAs, 150 ppm of VE, and 0.5 ppm of Se) additionally supplemented with xanthophylls (lutein and zeaxanthin) featured higher productivity, better FCRs, higher concentrations of carotenoids and VE in eggs, better yolk pigmentation in comparison to non-supplemented control without deterioration of egg morphology. The best results were found in group 4 which received 18 ppm of xanthophylls (as 900 ppm of “Biofon Yellow”). Egg production in this group was higher in comparison to control by 4.0%, egg mass output was higher by 4.3%, concentration of total carotenoids per 100 g of edible parts of eggs was 4.0-fold higher, VE was higher by 13.5%, Se – by 0.2%, ω-6 and ω-3 PUFAs – by 18.8 and 20.0%, respectively (at ω-6/ω-3 ratio 3.1:1). FCRs per 10 eggs and per 1 kg of eggs was lower by 3.1 and 2.8%, respectively. The increase in the concentration of total carotenoids in yolk resulted in 1.8-fold higher intensity of yolk coloration improving the market appearance and consumer attractibility of the eggs.

Acknowledgements
The study was financed by the Russian Science Foundation, grant No 16-16-04047.

References
[1] Perić N, Rodić V and Milošević N 2011 Production of poultry met and eggs as functional food – Challenges and opportunities Biotechnology in Animal Husbandry 27 (3) 511-520
[2] Siry I, Kápolna E, Kápolna B and Lugasi A 2008 Functional food. Product development, marketing and consumer acceptance - A review Appetite 51 (3) 456-467
[3] Shishkina D I and Sokolov A Y 2018 Analysis of foreign technologies for the functional meat products Proceedings of the Voronezh State University of Engineering Technologies (In Russ.) 80 (2) 189-194
[4] Lavrenova Z I and Nazarova N E 2018 Development of technology for the production of poultry products prophylactic purpose (smoked-baked galantine from chicken meat with Bulgarian pepper). Proceedings of the Voronezh State University of Engineering Technologies (In Russ.) 80 (3) 272-277
[5] Surai P 2010 How to improve the egg nutritive value Compound Feeds 6 95-96
[6] Panaite T D, Nour V, Vlaicu P A, Ropota M, Corbu A R and Saracila M 2019 Flaxseed and dried tomato waste used together in laying hens diet Archives of Animal Nutrition 73 (3) 222-238
[7] Surai P 2016 Enriched eggs: fictions and realities Feeds & Facts 4 4-8
[8] Singh V P, Pathak V and Akhilesh K V 2012 Modified or enriched eggs: a smart approach in egg industry: a review American Journal of Food Technology 7 (5) 266-277
[9] Kavtarashvili A Sh, Stefanova I L and Svitkin V S 2019 Functional egg production. III. The role of carotenoids Agricultural Biology 54 (4) 681-693
[10] Saksonova E O 2005 Luthein and zeaxanthin as main components of antioxidative eye protection system Russian Medical Journal 13 (2) 124-129
[11] Ham W T, Mueller A, Ruffolo J J, Millen J E, Cleary S F, Guerry R K and Guerry D 1984 Basic mechanisms underlying the production of photochemical lesions in the mammalian retina Current Eye Research 3 (1) 165-174
[12] Khachik F, Bernstein P S and Garland D L 1997 Identification of lutein and zeaxanthin oxidation products in human and monkey retinas *Investigative Ophthalmology & Visual Science* **38** (9) 1802-1811

[13] Kavtarashvili A Sh, Stefanova I L, Svitkin V S and Novotorov E N 2018 The ration recipes developed to improve effective and safe biofortification of hen (*Gallus gallus* L.) eggs *Agricultural Biology* **53** (4) 787-798