AGRICULTURAL BIOLOGY,
2018, V. 53, № 4, pp. 787-798
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.5:636.084.524

doi: 10.15389/agrobiology.2018.4.787eng
doi: 10.15389/agrobiology.2018.4.787rus

THE RATION RECIPES DEVELOPED TO IMPROVE EFFECTIVE AND SAFE BIOFORTIFICATION OF HEN (*Gallus gallus* L.) EGGS

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The authors declare no conflict of interests
Acknowledgements:
Supported financially by Russian Science Foundation, grant No 16-16-04047
Received March 1, 2018

Abstract

Enrichment of chicken eggs with ω-3 polyunsaturated fatty acids (PUFAs) is relevant worldwide, but scientists and practitioners face certain challenges. Effective biofortification requires dietary source of ω-3 PUFAs bioavailable for laying hens that will not compromise livability, health, and productivity of layers. Since any increase in ω-3 PUFA level in dietary lipids can deteriorate the oxidative stability of egg lipids causing faster quality loss and emergence of fishy odor and taste in stored and/or cooked eggs, dietary antioxidants should be used of which vitamin E and selenium (Se) are the most effective. High costs of the ω-3 PUFA-enriched diets for layers should also be diminished. The most popular source of dietary ω-3 PUFAs for layers is flax (seed, oil, cake); however, its dietary level should not exceed 15 % even when used with the appropriate multi-enzyme preparations. This paper is the first to report on comparative study of low-cost feed recipes that we suggest for effective concurrent enrichment of eggs with ω-3 PUFAs, vitamin E, and Se with no negative impacts on layers' livability and productivity. The trials were performed in 2016-2017 at the Zagorskoye Center for Genetics & Selection (Moscow Province) on SP 789 cross layers from 140 to 200 days of age. In the Trial 1 the diets for layers were supplemented with flaxseed oil (3 %) and cake (5 or 10 %), synthetic vitamin E (DL-α-tocopherol, 100 or 150 ppm), and Se preparations Sel-Plex® (Alltech, USA), DAFS-25 (Russia), and sodium selenite (0.5 ppm of Se). These doses of the additives led to a 4.5-4.7-fold increase in ω-3 PUFA level, a 1.9-3.6-fold increase in vitamin E and a 1.5-2.2-fold increase in Se content in the edible parts of eggs. Additionally, egg production was 0.6-4.0 % higher, total egg weight was 0.3-8.4 % higher, and feed conversion ratio was improved by 4.1-9.4 %. In Trial 2 layers were fed with optimized doses of the additives determined in the previous trial (flaxseed oil 3 %, flaxseed cake 5 %, vitamin E 150 ppm, Se 0.5 ppm); the comparative efficiency of different Se sources (Sel-Plex®, Sel-Plex® + DAFS-25 at 1:1, Sel-Plex® + sodium selenite at 1:1) and organic vitamin E vs. synthetic vitamin E preparation was studied. The combinations of Sel-Plex® with other Se sources and the substitution of organic vitamin E for the synthetic source improved egg production, egg weight, feed conversion ratio, and decreased diet cost. The best results were found for the mixture of Sel-Plex® and selenite and an organic source of vitamin E. In this, the content of total ω-3 PUFAs in eggs was 4.9 times higher compared to control (with ω-6/ω-3 PUFAs ratio 2.3:1 vs. 14.2:1 in control), the contents of individual ω-3 PUFAs were also significantly higher, i.e. 7.1-fold for α-linolenic acid, 1.8-fold for eicosapentaenoic acid, 3.2-fold for docosapentaenoic acid, and 3.8-fold for docosahexaenoic acid. The content of vitamin E in the eggs was 2.8 times higher, Se level was 2.2 times higher. In this trial, the egg production improved by 10.1 % compared to control, egg weigh output per layer was 13.2 % higher. Feed expenses per 10 eggs lowered by 7.6 %, and per 1 kg of egg weight by 9.9 %. Total diet costs were 1.2 % lower.

Keywords: chicken, functional eggs, ω-3 polyunsaturated fatty acids, selenium, vitamin E, diet cost

Market of functional products increasing the supply of all necessary nutrients to the consumers and promoting the disease prevention has been growing strongly over the last few years [1-3]. Products rich in essential fatty acids, pri-

marily ω-3 polyunsaturated fatty acids (PUFAs), i.e. α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which cannot be synthesized in sufficient amounts by animals and birds, are of particular interest [4]. Humans need these substances for the development of brain, visual function and for prevention of cardiovascular diseases, etc. [5]. The optimum ratio of ω-6/ω-3 PUFA in human diet is still debatable. Opinions vary from 1:1, pleading the fact that the two PUFA groups compete with each other in metabolic processes [6], and to 2-3:1 [7]. In developed countries, this ratio is remarkably high. Estimates vary from 10:1 to 25:1 [8].

Current diets of layers based on corn and other grains are leaning heavily toward ω-6 PUFA (with small contents of ALA and virtually non-existent DHA and EPA) [9]. Vegetable oils (corn, soy, sunflower, rape) used in the diets also contain considerable amounts of ω-6 PUFA (mostly linolenic acid, LA) and very little ω-3 PUFA [10], excluding however certain species of rape [11]. With this kind of diet, ω-3 PUFA content in the egg is small and the ω-6/ω-3 PUFA ratio is way above the optimum value [12].

As the most available source of complete protein and fatty acids, chicken eggs represent a food product that is most suitable for added functional properties [13]. Egg white covers approx. 12% of a daily demand of a human body and, therefore, is considered to be a model complete protein. Egg yolk contains saturated, monounsaturated and polyunsaturated fatty acids (approx. 7 % of a daily demand of a human body, including approx. 11% of essential fatty acids) [7]. Amino acid composition of a chicken egg virtually does not depend on the diet of the layer [13], whereas lipid composition largely depends on the lipid profile of the diet [4, 14]. Thanks to high-speed and adaptable metabolism, biologically active substances take 2-4 weeks to transit from the layer's diet to eggs, so one can vary the composition of the egg yolk by modifying the diet of the layer [4, 15, 16]. However, enriched diets may have negative impact on physiological condition of the birds and boost the cost of products (by 15-20 % for functional poultry products on retail market) [1, 17].

Enrichment of eggs with ω-3 PUFA requires a proper source where high biological availability of this fatty acid would have little negative impact on the egg production, layers' health and quality of eggs, including organoleptic parameters. One should control the ω-6/ω-3 PUFA ratio both in the diet and in the obtained eggs. Increase in the share and extent of PUFA (including ω-3 variety) undersaturation in the diet will reduce oxidation stability [18, 19] and cause toxic oxidation products to occur in the body of the layer and to carry over into the eggs thereby compromising their quality during storage and/or cooking [20]. In addition, enrichment of eggs with ω-3 PUFA often results in unpleasant fishy odor [21].

ω-3 PUFA in the layers' diet mostly comes from flax products (seed, oil, cake) wherein ALA contents is higher than in any other oil crops: ALA comprises over 50% of lipids in flax seed [22]. Flax oil is less frequent in the diet of the poultry than seed or cake due to insufficient availability and relatively high cost of this product [23]. Flax products diet, however, causes the odor of spoiled fishy in the eggs (probably, due to oxidation of PUFA-enriched lipids). Whole flax seed contains approx. 40% of fat, 20-25% protein and 3-10% adhesive substances (held mostly in seed coats), which, along with lignan phytoestrogens , linatine (pyridoxine antagonist) and linamarin (cyanogenetic glucoside), are deemed to be major anti-nutritional factors of flax [24]. Generally recommended diet of a grown bird should contain up to 10% of flax seed [23] which should be ground, autoclaved, granulated, extruded, subjected to microwave thermal treatment, etc., to enhance availability of protein and ω-3 PUFA [25, 26]. Phytoestrogens in flax

seed negatively impact egg and yolk weight, and adhesive substances reduce the egg-laying capacity. Arabinoxylanic fraction of the adhesive substance in the seed vastly increases chymus viscosity, thereby reducing the effect of dietary nutrients [27]. The diets with rape should be enriched with ferments capable of breaking the non-starch polysaccharides [28, 29]. There are few similar studies known in the context of flax. There were reports confirming the improved egg-laying capacity and feed conversion rate if the layers were fed 15% of flax seed with multienzyme complex Superzyme®-OM (Canadian Bio-Systems, Inc., Canada) that helps break the non-starch polysaccharides (at the same dosage of flax seed without enzyme additives, egg-laying capacity and shell mass went down compared to control without flax seed and enzyme additives). Enzyme application would increase total ω-3-PUFA (from 546 mg to 578 mg per 1 egg of 60 g) and DHA (from 91.8 mg to 101.9 mg) (30).

Oxidation stability of PUFA-enriched lipids is increased by antioxidants added to the feed, e.g. vitamins E, A and C, carotenoids, Se, I, etc., which also are valuable bioactive agents. It would partially solve the problem of fishy odor in eggs. One should also use fresh feed with minimum-oxidation lipids [20, 31, 32]. At current prices on quality feed sources of ω-3-fatty acids, Western producers of FA-enriched eggs spend almost twice as much as on non-enriched eggs [7], so antioxidants without ω-3-fatty acids appear to be a feasible alternative.

We are the first to study the potential of biofortification of edible eggs by vitamin E, selenium and ω-3 PUFA in Russian cross breed chickens. This paper gives the dosages we established for flax cake, oil, fatty acids, organic source of vitamin E, Sel-Plex® and sodium selenite, which increase egg-laying capacity and egg weigh output per layer without negative physiological effect, while cutting the feed consumption per unit of product and the costs for compound feed.

This paper is dedicated to the study of efficiency of simultaneous enrichment of edible chicken eggs with ω-3 polyunsaturated fatty acids, vitamin E and selenium with the help of feed additives, and to assessment of impact of the latter on health and productivity of poultry.

Methods. Trials were held in the animal facility Zagorskoye Center for Genetics and Selection (Sergiev Posad, Moscow Region) in 2016-2017 on SP 789 cross layers (*Gallus gallus* L.) aged 140 to 200 days and held in poultry cages (5 layers in each cage) at intermittent lighting 2C:5T:3C:2T:3C:9T.

Six gropus, each comprising of 30 layers, were formed for determination of necessary dosage of ω-3 PUFA, vitamin E and selenium by the analogue method. Group I (control) was given standard diet (basic diet, BD) comprising of wheat (56.1%), bran (11.1 %), soy bean meal (9.3 %), sunflower cake (9.3 %), sunflower oil (3.0 %); ω-6 and ω-3 PUFA content is 3.62% and 0.14% respectively, their ratio is 25.9:1; synthetic vitamin E (DL-α-tocopherol) content is 10 g, pure selenium (sodium selenite as a source) makes 0.2 g/t of compound feed. In test groups II and III, sunflower oil in BD was totally replaced by flaxseed oil (3 %), cutting the share of wheat, soy bean meal, sunflower cake and wheat bran down to 55.7; 7.0; 8.2 and 9.9 respectively, with adding 5% of flaxseed cake. In test group II, selenium came from preparation Sel-Plex® (Alltech, USA), in test group III the Se source was DAFS-25 (LLC Sulfat, Russia). In the diet of test groups II and III, the amount of ω-6 and ω-3 PUFA was 2.09% and 1.97% respectively, at 1.06:1; vitamin E (DL-α-tocopherol) was 100 g/t, pure selenium was 0.5 g/t of compound feed. In test groups IV-VI, sunflower oil was also replaced with flaxseed oil, with reducing the content of soy bean meal, sunflower cake and wheat bran to 4.7%. 7.1% and 8.9% respectively, and adding 10% flaxseed cake to the diet. Test groups IV, V and VI were was fed Sel-Plex®, DAFS-25, and sodium selenite, respectively. In these diets, ω-6 and ω-3 PUFA content

was 1.82% and 2.35% respectively (at 0.77:1) with 150 g vitamin E (DL-α-tocopherol) and 0.5 g pure selenium per ton of compound feed. Diets of all groups were supplemented with 0.01% enzyme preparations Feedbest W (xylanase and β-glucanase) and Feedbest P (3-phytase) (LLC Sibbiofarm, Russia).

Seven groups, each comprising of 30 layers, were formed for determination of complex enrichment of edible chicken eggs with ω-3 PUFA, non-organic and organic forms of vitamin E and selenium by analogue method. Group I (control, BD) was fed wheat (57.2%), bran (5.5%), soy bean meal (10.4%), sunflower cake (8.6%), corn gluten (3.0%), sunflower oil (4.0%). Content of ω-6 and ω-3 PUFA was 3.69% and 0.12% respectively (at 30.8:1), vitamin E (DL-α-tocopherol) is 10 g/t, pure selenium (source: sodium selenite) is 0.2 g/t compound feed. In test groups II-IV, 3% sunflower oil was replaced with flaxseed oil, content of wheat, bran and soy bean meal was reduced to 56.8%; 4.3% and 6.5% respectively; sunflower cake increased to 9.1% and 5% of flaxseed cake added. In test groups II, III and IV selenium came from Sel-Plex®, Sel-Plex® and DAFS-25 (1:1), and Sel-Plex® and sodium selenite (1:1). Content of ω-6 and ω-3 PUFA in the diets is 2.49% and 2.16% respectively (at 1.15:1), with 150 g/t vitamin E (DL-α-tocopherol) and 0.5 g/t pure selenium. In test groups V-VII, sunflower oil (4%) in BD was replaced with flaxseed oil (3%) in combination with fat production waste (a substrate containing over 90% fats, 280 μg/g natural carotenoids and over 11300 μg/g α-tocopherola, the organic form of vitamin E) (1.5%), with adding flaxseed cake (5%), reducing bran and soy bean meal (down to 2.64% and 6.53% respectively) and increasing sunflower cake contents (up to 9.63%). Se-source in test groups V, VI and VII was Sel-Plex®; Sel-Plex® and DAFS-25 (1:1); and Sel-Plex® and sodium selenite (1:1). Content of ω-6 и ω-3 PUFA was 2.50% and 2.23% (at 1.12:1), with 150 g/t organic vitamin E (D-α-tocopherol) and 0.5 g/t pure selenium. Diets of all groups were supplemented with enzyme preparation Record (100 g/t feed; CJSC Ferment, Belarus).

Livability of population was controlled on a daily basis, live weight control involved individual weighing of the entire population aged 140 and 200 days. Egg production per laying hen was estimated by the number of eggs laid by each group; egg weight by individual weighing of each egg the hens laid within 3 consecutive days in the middle of each month; output per hen (weight) — by the number of eggs laid and average weight of the eggs for each group; output per hen for category — by weighing and visual inspection of the eggs laid by the hens within 3 consecutive days in the middle of each month (national standard of the Russian Federation GOST 31654-2012 “Edible Chicken Eggs. Specifications”). Feed consumption was estimated by daily control of the feed served and feed stock available at the end of each week; feed consumption per 10 eggs and per 1 kg of egg weight was calculated on the basis of data on feed consumption, egg production and egg output (by weight). Weight of the egg white, egg yolk and egg shell was determined by generally accepted methods in the middle of each month; contents of ω-3 PUFA, vitamin E and selenium in the yolk and in the egg white were quantified on days 30 and 60 days of examination.

Se weight fraction was measured by electrothermal atomic absorption spectrometry (spectrometer Duo 240 FS/240Z, Varian, USA). Samples were decomposed by microwave sample preparation system Milestone START D (Milestone Systems, Italy). Weight fraction of vitamin E was measured by high-performance liquid chromatography (chromatographic system Knauer, Knauer Engineering GmbH Industrieanlagen & Co., Germany). Samples were prepared by the commonly accepted alkaline saponification method followed by extraction with diethyl ether. Weight fraction of crude fat was measured using the Randall method and extractor VELP Ser148 (VELP, Italy); fatty acids composition was

studied by capillary gas-liquid chromatography using gas chromatograph Kristall-2000M (Chromatech, Russia). Methyl ethers of fatty acids were separated in capillary column Stabilwax®-DE (Restek, USA) (l = 60 m, internal Ø 0.32 mm, film thickness 0.5 mm) and registered by flame-ionization detector Kristall-2000M (CJSC SBC Chromatech, Russia).

Data processing proceeded by variation statistics in Microsoft Excel. Mean values (*M*) and standard error of mean (±SEM) are set forth in the tables. Reliability was estimated by the Student *t*-test. Variations were considered statistically significant at $p < 0.05$.

Results. The purpose of Test 1 was to estimate the dose of sources of ω-3 PUFA, vitamin E and selenium in the diet of layers. Over the 60-day period, livability of the flock in every trial group was 100% (Table 1). Live weight of layers aged 140 days was virtually the same in groups I-VI. However, the biggest live weight among the layers aged 200 days was in group II (1.4%-3.6% higher than in the remaining groups). Group III yielded the worst results, 0.9% below the control group. In terms of egg-laying capacity, groups II, III, V and VI were way ahead of the others with the negligible difference between them. Group I demonstrated the lowest egg-laying capacity, 0.6%-3.9% behind the others. Group II had the highest mean weight of the egg, mean egg weight output per layer, as well as the output of super and grade 1 eggs (respectively 4.5%-6.7%; 5.0%-8.4%; 3.7%-9.7% and 11.0%-23.8% higher than in the remaining groups). Group V yielded the smallest egg weight, while the control group yielded the smallest egg weight output. Tendency toward the drop in the egg weight was in the poultry receiving diet with 10% of flaxseed cake and pure selenium (0.5 g/t) in DAFS-25. The difference in weight of eggs was significant between group II and groups I, III-VI ($p < 0.001$). In recent studies [33], addition of 10% flaxseed cake to the diet increased egg production by 4% ($p < 0.05$), with the egg weight considerably lower than in control group. According to another report [34], increase in vitamin E content from 100 to 200 g/t boosted egg laying ($p < 0.01$) while the egg weight dropped ($p < 0.01$).

1. Body weight, egg production, egg quality and feed consumption in SP 789 cross layers fed with varying diets including ω-3 polyunsaturated fatty acids, vitamin E and selenium (*M*±SEM, Zagorskoye CGS, Sergiev Posad, Moscow Region, 2016-2017)

Indicator	Group (n = 30)					
	I (control)	II	III	IV	V	VI
Live weight, g:						
age 140 days	1307±16.42	1308±17.93	1312±11.31	1309±15.31	1308±13.60	1318±16.01
age 200 days	1616±26.29	1658±26.28	1601±27.72	1620±38.28	1613±22.16	1635±24.95
Egg production per layer, pcs.	47.5	49.2	49.0	47.8	49.3	49.4
Egg laying intensity, %	79.2	82.0	81.7	79.7	82.2	82.3
Mean weight of an egg, g	56.2±0.50	58.7±0.43*	55.4±0.49*	55.8±0.48	55.0±0.46	55.9±0.48*
Egg output by grade, %:						
Premium	2.04	1.94	1.97	1.32	1.35	1.97
Super (0)	3.40	10.97	5.92	7.24	1.35	1.32
1	50.34	61.29	37.50	43.42	45.95	48.03
2	40.14	21.94	49.34	44.08	48.97	45.39
3	0.68	0.64	1.32	1.31	0.67	0.66
breakage and cracks	3.40	3.22	3.95	2.63	2.70	2.63
Egg weight output per layer, kg	2.675	2.900	2.722	2.683	2.717	2.761
Feed consumption:						
per 1 hen/day, g	109.2	107.0	106.7	103.1	104.5	106.5
per 10 eggs, kg	1.38	1.31	1.31	1.29	1.27	1.29
per 1 kg of egg weight, kg	2.45	2.22	2.35	2.31	2.31	2.31
Cost per 1 t compound feed, RUB	14098	14504	14282	14374	14151	14104

N o t e. Groups and accounting methods (Test 1) are described in section Techniques.
* Difference with control group is statistically significant at $p < 0.001$.

The lowest feed consumption per hen for day was in group IV (1.3%-5.6% below the other groups), while group I (control) kept the maximum feed

consumption. The cost of feed per 10 eggs was the lowest in group V, and in terms of 1 kg of egg weight in group II (respectively 1.6%-8.0% and 3.9%-9.4% smaller than in the remaining groups). These indicators were the highest in control group where minimum egg production and maximum feed consumption per hen for day were recorded.

2. Morphological and chemical characterization of eggs laid by SP 789 cross layers fed various diets including ω-3 polyunsaturated fatty acids (PUFA)**, vitamin E and selenium** (*M*±SEM, Zagorskoye CGS, Sergiev Posad, Moscow Region, 2016-2017)

Indicator	Group (n = 30)					
	I (control)	II	III	IV	V	VI
Egg yolk weight:						
absolute, g	14.27±0.44	14.23±0.32	13.99±0.31	13.69±0.34	14.20±0.30	14.49±0.34
relative, %	23.95	23.63	24.44	23.38	24.70	24.18
Egg while weight:						
absolute, g	39.00±0.56	39.62±0.66	37.1±0.57*	38.54±0.46	37.08±0.49*	38.94±0.60
relative, %	65.45	65.79	64.80	65.80	64.51	65.00
Egg shell weight:						
absolute, g	6.32±0.10	6.37±0.13	6.16±0.08	6.34±0.10	6.20±0.09	6.48±0.08
relative, %	10.60	10.58	10.76	10.82	10.79	10.82
Contents per 100 g of the edible part of egg (bulk samples):						
Se, μg	28.3	61.6	58.2	61.8	58.9	43.1
vitamin E, mg	2.31	4.47	5.04	6.40	8.35	5.50
ω-6 PUFA, mg	2181	1630	1504	1535	1321	1410
ω-3 PUFA, mg	172	767	767	807	767	796
including						
α-linolenic, mg	69	539	537	547	577	515
ω-3-eicosapentaenoic, mg	12	22	26	25	28	29
ω-3-docosapentaenoic, mg	8.5	29	30	31	31	29
ω-3-docosahexaenoic, mg	64	148	146	174	152	144
ω-6/ω-3 PUFA ratio	12.7:1	2.1:1	2.0:1	1.9:1	1.7:1	1.8:1

N o t e. Groups and accounting methods (Test 1) are described in section Techniques.
* Difference with control group is statistically significant at $p < 0.05$.

According to morphological and chemical study of the eggs (Table 2), the groups did not differ too much in terms of the absolute and relative weight of the egg yolk over the trial period (60 days). Maximum absolute weight of the egg white was in group II (1.5%-6.9% above the other groups). The difference in this value proved to be significant between groups I and III, V ($p < 0.05$); II and III, V ($p < 0.01$); VI and III, V ($p < 0.05$); IV and V ($p < 0.05$). In terms of the relative weight of egg white, the leading groups were group II and IV where Se source was Sel-Plex®, i.e. the weight of eggs in these groups grew due to egg white. There were no considerable differences between the groups for absolute and relative weight of the egg shell.

In groups II-VI, vitamin E content in 100 g of the edible part of egg was 1.9-3.6 times higher than in the control. Increasing the dose of vitamin from 100 g/t to 150 g/t compound feed naturally increased its content in the egg itself. Similar correlation was observed in other studies [34, 35], i.e. vitamin E content in the egg directly depended on the dose thereof in the diet.

Selene content in 100 g of the edible part of egg in the test groups was 1.5-2.2 higher than in the control. Of all test groups, this value was the lowest in group VI where sodium selenite was used. Our data correlate with the findings of other authors [36] who report that selenium content in egg white and egg yolk depends on the dosage and the form of selenium in the layers' diet.

Deposition of ω-3 PUFA per 100 g of the edible part of egg in test groups was 4.5-4.7 times higher compared to control, with 7.5-8.4-fold α-linolenic acid, 1.8-2.4-fold eicosapentaenoic acid, 3.2-3.4-fold docosapentaenoic acid, and 2.3-2.7-fold docosahexaenoic acid. The ω-6/ω-3 PUFA ratio in groups II and VI was 1.7-2.1:1 vs. 12.7:1 in control. It should be noted that add-

ing 5% flaxseed cake (groups II and III) and 10% flaxseed cake (groups IV-VI) in combination with 3% flaxseed oil have very little impact on ω-3 PUFA in eggs, whereas decrease in ω-6 to ω-3 PUFA ratio occurs due to decreasing accumulation of ω-6 PUFA. The test groups where DAFS-25 was the Se source (III and V) and sodium selenite (VI) show a tendency toward decrease of ω-6/ω-3 PUFA ratio. These data correlate with the findings of N. Gjorgovska et al. [34] who reported an increase in the content of these fatty acids in the eggs of chickens fed with a higher dose of ω-3 PUFA (DHA and EPA). Fresh eggs and the eggs from control hens and groups II-III stored for 25 days at room temperature had no foreign odor or flavor. Fresh eggs from groups IV-VI had no foreign odor or flavor, too, but when these eggs were stored, a slight fishy odor and flavor emerged before and after cooking.

Complex enrichment of chicken eggs with ω-3 PUFA, selenium and vitamin E during the first test increased the cost of feed in groups II-VI by 0.04%-2.88% compared to the control group. That is why in the next experiment we endeavored to obtain similar results for biofortification of edible eggs without causing the increase in cost of the compound feeds.

In Test 2 (Table 3), livability in each group was also 100%. No significant difference in live weight of hens aged 200 days was detected. The highest productivity and egg weight output per layer was in group VII (2.4%-10.1% and 3.0%-13.2% higher than in other groups). Control group yielded the lowest values. Following the replacement of synthetic source of vitamin E with organic (fatty acids), mean egg weight increased in groups V-VII by 0.6-0.8 g or by 1.1%-1.4%, with group VII dominance. The lowest egg weight was in the control group, 0.6-1.6 g (or 1.1%-2.8%) lower than in other groups. Difference in these values between groups V-VII and group I was significant ($p < 0.05$). Available literature provides no data regarding the impact of any such fatty acid preparations on the effective accumulation of vitamin E in eggs.

3. Body weight, egg productivity, egg quality and feed consumption with SP 789 cross layers following optimization of diet by inclusion of ω-3 polyunsaturated fatty acids, non-organic and organic forms of vitamin E and selenium (*M*±SEM, Zagorskoye CGS, Sergiev Posad, Moscow Region, 2016-2017)

Indicator	Group (n = 30)						
	I (control)	II	III	IV	V	VI	VII
Live weight, g:							
age 140 days	1372±19.87	1370±22.68	1392±21.24	1396±23.51	1379±20.08	1366±22.37	1390±34.89
age 200 days	1543±22.87	1592±27.18	1615±32.28	1597±34.90	1602±30.08	1577±27.47	1566±29.76
Egg production per layer, pcs.	46.5	48.6	49.0	49.2	49.6	50.0	51.2
Egg laying intensity, %	77.6	81.0	81.7	82.1	82.7	83.3	85.4
Mean weight of an egg, g	54.9±0.48	55.5±0.44	55.6±0.40	55.7±0.36	56.1±0.34*	56.2±0.40	56.5±0.43*
Egg weight output per layer, kg	2.555	2.691	2.725	2.757	2.784	2.808	2.893
Feed consumption:							
per 1 hen/day, g	111.7	111.9	111.7	112.5	114.8	114.1	113.6
per 10 eggs, kg	1.44	1.38	1.37	1.37	1.39	1.37	1.33
per 1 kg of egg weight, kg	2.62	2.50	2.46	2.45	2.47	2.44	2.36
Cost per 1 t compound feed, RUB	14863	14965	14878	14855	14827	14711	14688

N o t e. Groups and accounting methods (Test 1) are described in section Techniques.
* Difference with control group is statistically significant at $p < 0.05$.

The lowest feed consumption rates (per 1vhen/day) were in groups I, II and III. Group V had the highest consumption (2.8% higher than control group). The lowest feed consumption per 10 eggs and 1 kg of egg weight (respectively 2.9-7.6 and 3.3%-9.9% smaller than in other groups) was in group VII where the maximum productivity and egg weight output per layer were observed.

Cutting the content of Sel-Plex® by half and balancing the diet with DAFS-25 in groups III and VI reduced the costs per 1 t compound feed by RUB 87 and RUB 116 (or 0.58% and 0.78%) respectively; and replacement of

50% of Sel-Plex® with sodium selenite in groups IV and VII reduced the costs per 1 t feed by RUB 110 and RUB 139 (or by 0.74% and 0.94%), respectively, as compared to groups and V. Replacement of the synthetic source of vitamin E with organic one (fatty acids from oil and fat production waste) in groups V-VII reduced the costs per 1 t compound feed by RUB 138-167 (or by 0.92%-1.12%) vs. groups II-IV. Group VII showed the lowest results, by RUB 175 (or 1.18%) lower than in control group and by RUB 23-277 (0.16%-1.85%) below groups II-VI.

The groups do not differ significantly in absolute and relative weight of the egg yolk, white and shell (Table 4). Se concentration in 100 g of the edible part of the egg in groups II-VII was 2.2-2.3 times, and vitamin E — 2.0-2.8 times higher than in the control group. Test groups show virtually similar Se content. A higher vitamin E concentration per 100 g of the edible part of egg was in group IV with its synthetic source and in group VII with its organic source. Accumulation of ω-3 PUFA per 100 g of the edible part of egg in groups II-IV was 3.4-5.0 times higher than in the control group, with 5.2-8.0-fold α-linolenic acid, 1.4-2.4-fold eicosapentaenoic acid, 1.5-3.2-fold docosapentaenoic acid, and 2.3-3.8-fold docosahexaenoic acid levels. Ratio ω-6 ω-3 PUFA in groups II-IV was 2.3-2.9:1 against 14.2:1 in control group. It should be noted that introduction of either synthetic or organic vitamin E in the feed caused the highest content of ω-3 and ω-6 PUFA per 100 g of the edible part of egg in groups where selenium sources were Sel-Plex® with sodium selenite in proportion 1:1 (groups IV and VII).

4. Morphological and chemical characterization of eggs laid by SP 789 cross layers fed optimized diets including ω-3 polyunsaturated fatty acids, non-organic and organic forms of vitamin E and selenium (*M*±SEM, Zagorskoye CGS, Sergiev Posad, Moscow Region, 2016-2017)

Indicator	Group (n = 30)						
	I (control)	II	III	IV	V	VI	VII
Egg yolk weight:							
absolute, g	12.38±0.31	12,58±0,32	12,56±0,28	12,64±0,34	12,72±0,39	12,80±0,32	12,89±0,43
relative, %	22.55	22,67	22,59	22,69	22,68	22,78	22,81
Egg while weight:							
absolute, g	36.32±1.15	36,77±1,18	36,81±1,04	36,89±0,97	37,16±0,97	37,15±0,95	37,34±1,02
relative, %	66.16	66,26	66,20	66,24	66,23	66,10	66,09
Egg shell weight:							
absolute, g	6.20±0.21	6,14±0,17	6,23±0,21	6,17±0,17	6,22±0,20	6,25±0,22	6,27±0,16
relative, %	11.29	11,07	11,21	11,07	11,09	11,12	11,10
Contents per 100 g of the edible part of egg (bulk samples):							
Se, μg	27.1	61,1	61,2	60,3	61,5	59,3	60,9
vitamin E, mg	3.10	6,16	7,04	7,95	8,19	8,59	8,82
ω-6 PUFA, mg	2717	2385	2312	2445	1915	2035	2148
ω-3 PUFA, mg	192	881	790	960	655	778	949
including							
α-linolenic, mg	77	618	502	581	400	490	544
ω-3-eicosapentaenoic, mg	11	25	19	26	15	19	20
ω-3-docosapentaenoic, mg	17	36	26	45	34	33	55
ω-3-docosahexaenoic, mg	87	202	243	308	206	236	330
ω-6/ω-3 PUFA ratio	14.2:1	2,7:1	2,9:1	2,5:1	2,9:1	2,6:1	2,3:1

N o t e. Groups and accounting methods (Test 1) are described in section Techniques.

According to P. Weill et al. [37], the use of 5% dietary extruded flaxseed, as compared to standard feed, results in 3.8 times higher content of n-3 PUFA in edible eggs, including 2.4 times higher DHA. Findings of I.D. Bean et al. [38] also indicate a significantly (at $p < 0.001$) increased content of linolenic acid, DHA and total ω-3 fatty acids in the eggs of layers fed with flaxseed.

Therefore, simultaneous use of dietary ω-3 polyunsaturated fatty acids (PUFA), selenium and vitamin E in tested doses will boost content of these substances in edible eggs without negative impact on livability and productivity of hens. For complex enrichment of edible eggs with ω-3 PUFA, vitamin E and

selenium, one needs to use dietary flaxseed oil and cake (3% and 5% respectively, with adding enzyme preparation), 150 g/t vitamin E and 0,5 g/t selenium. Replacement of half-dose of Sel-Plex® with DAFS-25 and sodium selenite and synthetic vitamin E (DL-α-tocopherol) with its organic source (D-α-tocopherol) enhances productivity and total egg weight per layer, while cutting the feed consumption per unit of product and the costs. Best results are achieved when Se sources are Sel-Plex® and sodium selenite (1:1) and the organic source of vitamin E are fatty acids of fat production waste. This group 4.9 times exceeded the control group for ω-3 PUFA content per 100 g of the edible part of egg (with ω-6/ω-3 PUFA ratio 2.3:1 against 14.2:1 in control group), including 7.1-fold increase in α-linolenic acid, 1.8-fold increase in eicosapentaenoic acid, 3.2-fold increase in docosapentaenoic acid, 3.8-fold increase in docosahexaenoic acid, 2.8-fold increase in vitamin E, and 2.2-fold increase in selenium. Layers' productivity is 10.1% higher, egg weight output per layer is 13.2% higher, whereas feed consumption per 10 eggs and 1 kg of egg weight reduces by 7.6% and 9.9% respectively, and feed consumption reduces by 1.2%.

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