AGRICULTURAL BIOLOGY,
2017, V. 52, № 6, pp. 1094-1104
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

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

UDC 636.52/.58:637.4.04/.07

doi: 10.15389/agrobiology.2017.6.1094rus
doi: 10.15389/agrobiology.2017.6.1094eng

BIOFORTIFICATION OF HEN EGGS: VITAMINS AND CAROTENOIDS
(review)

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The authors declare no conflict of interests
Acknowledgements:
Supported financially by Russian Science Foundation, grant № 16-16-04047
Received June 14, 2017

Abstract

The dependence of the vitamin content in the egg from the content in the chicken feed represents the saturation curves (V.M. Kodentsova et al., 2005; K. Hebert et al., 2005; S. Leeson et al., 2004; A.L. Shtele, 2004; S. Grobas et al., 2002). The effectiveness of the various forms of vitamins for enrichment of chicken feed and the use for this purpose of herbal supplements had been analyzed (P. Mattila et al., 2004; P.H. Mattila et al., 2011; M. Hammershøj et al., 2010, J.A. Moreno et al., 2016). The optimal content of vitamins and carotenoids in the poultry feed results in an increase to the maximum level of vitamins and carotenoids in the egg that makes them a significant source of vitamins D, E, B and carotenoid for humans. One such egg can provide up to 40-50 % of the recommended daily intake of vitamins D, B_{12}, A, pantothenic acid, 30 % of vitamin E, 20 % of folate, 10 % of vitamin A, 12 % of vitamin B_2, and up to 30 % of the adequate level of lutein intake. The advantage of biofortification is biotransformation in the chicken's body of synthetic vitamins added to food into their natural form, which deprives the arguments of opponents of enrichment of food products with synthetic vitamins. Comparison of the addition of different forms of vitamins showed that D_3 in the diet more effectively increased the vitamin content in egg yolk (P. Mattila et al., 2004). If the chicken feed contains vitamin D only as $25OHD_3$, then vitamin D in the form of cholecalciferol may be completely absent in the yolk (P.H. Matvila et al., 2011). Irradiation of chicken with ultraviolet light or free-range farming in the natural sunlight may provide an original, safe and natural alternative to produce vitamin D-enriched eggs (A. Schutkowski et al, 2013; J. Kühn et al., 2014, 2015) and chicken meat without the risk of overdose of this vitamin. By increasing the content of lutein in the yolk of a chicken egg, the bioavailability of this carotenoid can be substantially increased as compared to plant sources (G.J. Handelman et al., 1999). The enrichment of eggs with vitamins meets the criteria for the fortified foods (V.M. Kodentsova et al., 2010). Increasing the level of all vitamins in hen diet resulted in a simultaneous increase in the content of all vitamins in eggs (H. Zang et al., 2011). Biofortification has clear advantages over the technological enrichment since synthetic vitamins received from feed are converted into natural ones in hen body. Biofortification of eggs with vitamins is one of the most promising strategies to increase consumption of vitamins for population (M.S. Calvoa et al., 2013).

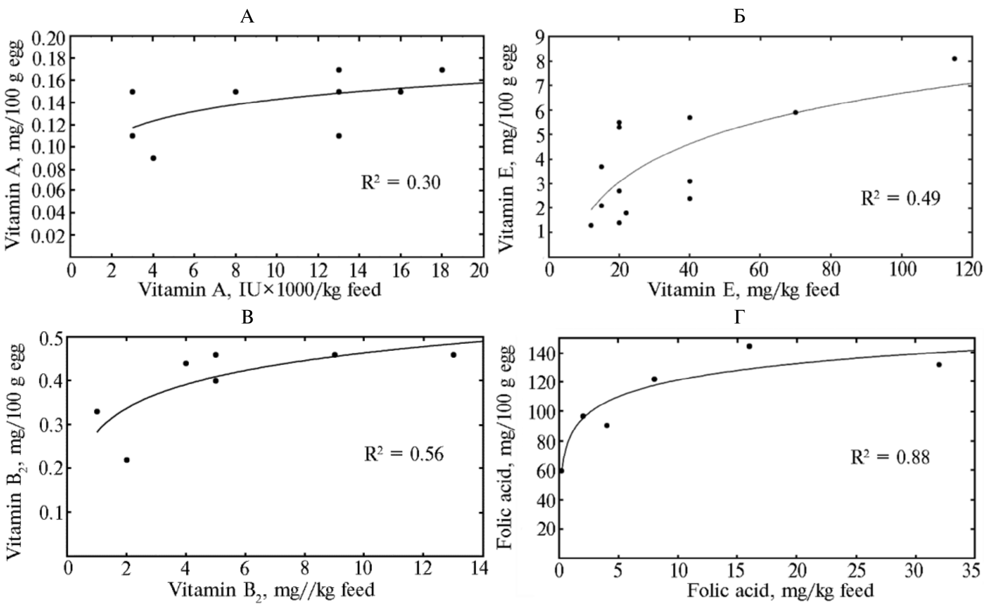
Keywords: biofortification, vitamins, carotenoids, poultry, eggs

The problem of micronutrient deficiency among population is being addressed in several ways. One of the approaches that find the world acceptance is technological modification, in which vitamins or their mixtures are added to raw material used in producing food products (e.g. bakery flour) or directly to food products for mass consumption (1). Unfortunately, an unreasonable opinion that synthetic vitamins are poorly metabolized in the body can be found not only among people, but also in scientific papers. Therefore, in recent years, more attention has been paid to biofortification (bio-addition) — the enrichment of livestock products via feed vitamin supplements. In this case, the vitamin, getting to the animal body passes through the biotransformation stages and is consumed by man in natural form. Although the laying hen need for most vitamins has been well studied and the optimal vitamin content in feed has been established, interest in the chicken egg as a perfect object to be used for improving its vitamin composition has increased in recent years, as the egg value can be increased in a natural way by enriching poultry ration with vitamins and minerals [2].

Chicken egg is a natural functional food of mass consumption which is used in nutrition of all population, so its biofiltration is of particular interest. According to the food nutritional tables, two large chicken eggs provide up to 20 % of the recommended daily protein intake, up to 30 % of vitamin B_2, up to 6 % of vitamin E, and 12 % of vitamin A [3, 4]. However, the real concentration of vitamins in eggs which were produced in different poultry farms and smallholder agricultures [5] depends on the vitamins in feeds and can differ essentially from the values indicated in the food chemical composition and nutritional tables [6, 7].

This review for the first time summarizes the modern findings on vitamin enhancing in the egg not only by enrichment of laying hen rations with vitamins, but also by herbal dietary components and UV irradiation of birds.

When vitamins content in the feed does not reach the norms recommended for egg-laying hens [2], the amount of vitamins in the egg is minimal (Fig.). An increase in most dietary vitamins in the poultry feed is accompanied by a gradual non-linear regression of its amount in the egg (see Fig.). It can substantially exceed the values given in the food nutritional tables used to calculate the intake of vitamins with a diet.



Vitamin A (A)**, E** (B)**, B_2** (C) **and folic acid** (D) **content in a sound egg depending on their dietary amounts** [5, 16-19, 25].

V i t a m i n E. The effect of various doses of vitamin E in laying hens diets, from 20-60 mg/kg feed [8] to 100-200 mg/kg feed [9, 10] and above [11], is shown on the Figure (see B). According to various researchers [12, 13], an increase in dietary vitamin E from 10 to 150 mg/kg was accompanied by its concentration in the egg yolk. This increasing was going on up to 200 mg of αtocopheryl acetate per 1 kg of feed [14]. The vitamin E accumulation in the egg yolk increased up to 6.8-38.9 % with the addition of selenium (0.1 g/t).

Amount of vitamins in eggs of laying hens fed with rations enriched with vitamins (literature data)

Vitamins	Data of national tables of the chemical composition of food products [6, 7]	Ration	
		without additional feed enrichment	with additional feed enrichment
D	88 IU 310 IU[a]	25-80 IU [17, 20] 150-450 IU[a] [21]	180-1700 IU [17] 33.7-150 μg[a] [21, 22]
K	–	0.01-0.05 mg [20]	0.05 mg [17]
B_6	0.14 mg 0.46 mg[a]	0.11-16 mg [17, 20] 0.35-1,50 mg[a] [17]	–
Niacin	0.19-0.20 mg	0.07-0.15 mg [17, 20, 23] 0.05 mg[a] [17]	–
B_{12}	0.0005 mg 0.0018 mg[a]	0.0004-0.0010 mg [17, 23] 0.0018 mg[a] [17]	0.0016 mg [17]
Folate	7-32 мкг 0.022 mg[a]	0.009-0.078 mg [2, 17, 23] 0.12-0.17 mg[a] [17, 23]	33-75 μg [24, 25]
Pantothenic acid	1.3 mg 4 mg[a]	1.2-1.6 mg [17, 26] 3.5-12.5 mg[a] [17, 20]	1.9 mg [17]
Biotin	0.02 mg 0.056 mg[a]	0.016-0.030 mg [17, 20] 0.1 mg[a]	0.070 mg [17]
β-Carotene	–	0.014 mg[a]	0.52 mg[a] [18]
Lutein	–	0.3 mg/60 g	1.5 mg/60 mg [27] 0.8-2.7 mg[a] [5]
Cryptoxanthin	–	–	0.08-0.20 mg[a] [5]

Note. Dashes mean the absence of data in accessible literature; [a] — the content per 100 gm of egg yolk.

Vitamin E is absorbed in the small intestine, and the effectiveness of this process depends on the diet composition, the used dosage, age, sex, and other individual chicken characteristics. Vitamin E accumulates in the liver and adipose tissue, but it is not enough for continuous requirements. For example, the amount of vitamin E that transfers from the laying hen in one egg is more than the reserve of this compound in the liver [12, 15].

The amount of dietary vitamin E affects the eggs quality, protects polyunsaturated fatty acids (PUFA) from oxidative damage for 28 days of storage at room temperature [28] and promotes retinol and carotenoids preservation [12, 15, 29, 30] in the egg. Enrichment of chicken feed with ω-3 PUFA together with vitamin E prolongs the best before dates, reducing the lipids oxidation [31]. According to A. Barroeta [32], at low PUFA content in chicken diet (15 g/kg), 60 mg/kg α-tocopherol should be imposed to the diet for maintaining lipid stability, whereas at high PUFA content (30 g PUFA/kg feed), 200 mg/kg is required. Vitamin E is non-toxic, even its high doses do not cause hypervitaminosis, but excessive addition to feed should be economically viable [33]. Furthermore, high doses of vitamin E (10000-20000 IU/kg of feed) significantly reduced the vitamin A and carotenoid concentration in the egg yolk [19].

In addition to tocopherols, natural sources of this vitamin are used for enrichment the chicken feed. Supplementation of rice bran oil (RBO) containing 1.3 % tocotrienols which are a form of natural vitamin E to the hen feed for 7 days improved their content up to 0.62 mg/egg against the usual content of 0.11 mg/egg [34].

Vitamin D. As compared to others, vitamin D comes not only with food, but it can also form in the skin of humans and animals under the influence of ultraviolet radiation, and in fact can not be denoted as a vitamin. It is a prohormone that converted to 1,25-dihydroxyvitamin D, its hormone form in the organism. There are from 200 up to 2000 genes directly or indirectly responding to the vitamin D action. The range of its proven physiological effect is extremely wide and includes inhibition of cell division, stimulation of insulin synthesis, apoptosis, suppression of renin production, stimulation of production of cathelicidin, the peptide having an antimicrobial effect, by macrophages [35]. Vitamin D deficiency is linked to many socially important chronic diseases (cardiovascular, myocardial infarction, type 2 diabetes, autoimmune diseases, tuberculosis, bronchial asthma, atopic dermatitis, urticaria, prostate, breast, intestinal cancers), neurocognitive disorders, and depressions [35].

The vitamin D pool in human is determined by the combination of its synthesis in skin under the influence of sunlight and the consumption of two basic alimental forms of vitamin D, the ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). A decrease in blood vitamin D concentration is observed in 50-92 % of the working age population and children of our country, independently of seasons [36]. The causes of vitamin D deficiency are both in inadequate food consumption, and in low endogenous synthesis due to the Russian geographical location [36]. Recommended amount of vitamin D_3 in the human body has an important preventive potential for health. Food sources of this vitamin for human is scarce, in addition vitamin D is not contained in products of vegetable origin. The main sources are (in descending content order) cod liver, fatty fish, chicken eggs, liver, butter.

Accordingly, the perspectives for the biofiltration of chicken eggs with vitamin D become particularly important [37]. Vitamin D is mainly located in the yolk in the forms of cholecalciferol and $25OHD_3$. The vitamin D egg content raises by its increasing in chicken feed. It is more effective to increase the vitamin egg yolk content by adding vitamin D3 to chicken diet [22]. According to some authors, it is possible to produce eggs with 2-fold vitamin D_3 concentration (approximately 2-3 μg/egg) [38] by redoubling vitamin D_3 feed content compared to physiological norm. The D_3 content in hen egg yolk which received 6,000 IU (150 μg) or 15,000 IU (375 μg) of vitamin D_3 per 1 kg of feed varied from 9.1 up to 13.6 μg/100 g and from 25.3 up to 33.7 μg/100 g, respectively. While adding dietary vitamin D_2, the indicator was 4.7-7.0 and 13.3-21.0 μg/100 g of yolk [22].

Since 2009, in the EU, according to EU Regulation No. 887/2009 [39], for chickens fattening, in addition to cholecalciferol, the stabilized metabolite of $25OHD_3$ is allowed. It is the main circulating (transport) form of the human's vitamin. The maximum content of $25OND_3$ combination with vitamin D_3 per 1 kg of chicken feed should not exceed 125 μg (5000 IU). But it was found, that if the chicken feed contains vitamin D only in the form of $25OHD_3$, it may be completely absent in the form of cholecalciferol in the yolk [38].

Alternatively, to get enriched vitamin D eggs, irradiation of chicken with ultraviolet light or bio-addition were suggested. The eggs of hens irradiated with ultraviolet for 3 hours daily during 4 weeks and provided D_3-adequate diet (3000 IU/kg of feed) contained 2.5 μg of vitamin D (vitamin D_3 + $25OHD_3$), which is almost 5 times higher compared to its amount in eggs of hens which were not exposed to ultraviolet light (dose at a distance of 20 cm was 76 $\mu W/cm^2$) [40]. Curiously, endogenous synthesis occurs mainly in the legs of chickens, where the plumage is the smallest. The reliance of the vitamin D_3 and $25OHD_3$ content in the egg yolk on the time of daily UV irradiation was nonlinear. With a daily irradiation for 300 min, vitamin D_3 content was increased to 28.6 μg/100 g of egg yolk dry matter, but did not reach the plateau, whereas the amount of $25OHD_3$ was maximal even after irradiation for 60 min [41]. Vitamin D_3 concentration in egg yolk was three- to fourfold higher ($p < 0.001$) in the hens that were exposed to sunlight under outdoor and indoor/free-range farming than in those kept indoor [42]. The vitamin D_3 concentration in egg yolk in the free-range farming was 14.3 μg/100 g dry matter, in contrast to 3.8 μg/100 g. The vitamin D content in the egg yolk under mixed keeping conditions occupied an intermediate position. The $25OHD_3$ content of egg yolk was also influenced by sunlight exposure, although it was less pronounced than that of the vitamin D_3 ($p < 0.05$).

Therefore, ultraviolet or natural sunlight irradiation of laying hens is a promising strategy to fortify eggs and chicken meat with vitamin D while at the same time providing a safe approach without risking an overdose of this vitamin.

Vitamin A and carotenoids. A 5.3-fold increase in the dietary vitamin A to the optimum content [2] resulted in an 1.3-fold increase in its content in the egg (see Fig., A).

The main carotenoids, which make natural egg yolk color, are lutein and zeaxanthin [43, 44]. Their content is up to 80-90 % of total carotenoids [2]. The maize-based diet of layer hens usually contains about 11.8 mg of carotenoids per 1 kg of feed (mainly in lutein and zeaxanthin), and a wheat-based diet contains 5.6 mg carotenoids per 1 kg [45]. Enhancing egg yolk color may result from adding dietary carrots, peppers, pumpkin, hips to 1 % of feed weight and herbal flour up to 5-6 %. Such enrichment allows for the carotenoids corresponding to 8-10 mg per 1 kg of feed [17]. Adding orange, yellow or purple carrots to the ration at 70 g per hen increased the yolk lutein more than 1.5-fold, and the yolk β-carotene more than 100-fold [46]. Eggs from hens, receiving a diet with cabbage *Brassica oleracea* var. *acephala* leaves (120 g per chicken for 24 hours) had a higher content of lutein, β-carotene, and orange xanthophyll violaxanthin [47]. Adding 5 or 10 grams of tomato powder per 1 kg diet increased amount of lycopene, β-carotene, lutein and vitamin A in the yolk, whereas malondialdehyde decreased [48]. The lutein content in egg yolk of Japanese quail increased significantly if the diet included 0.2 % of the calendula extract [49]. In the addition in fodder of 3 % chlorella powder, the amount of lutein increased from 0.20 mg per egg (13 μg/g yolk in the control group) to 0.43 mg per egg (27 μg/g yolk) [50]. Clearly, such egg carotenoid enrichment has fundamental importance in the production of organic foods. The example of obtaining a 'double biofortified egg' with an increased content of carotenoids by incorporating biofortified corn into the diet is described [51].

Besides the use of natural carotenoid sources for enhancing egg yolk pigmentation, different carotenoids including not specific for egg yolk are added to the poultry feed. Among them are Lucanthin (cantoxanthin) at a dose of 0.9-1.5 up to 70 mg/kg feed, and Lipocarotene, a mixture of β-carotene (0.9-1.8 g per thousand chickens) and lycopene (0.4-0.8 g per thousand chickens) [52]. Sometimes higher doses of carotenoids (up to 400 mg/kg of β-carotene, canthaxanthin and lutein) [53] are used. β-Carotene enrichment of chicken eggs at a dose of 200 mg/kg feed leads to rising pigment content in the yolk 37 times more, from 0.14 up to 5.2 μg/g [18]. Consequently, dietary carotenoids fed to laying hens leads to an increase in the total content of these pigments in eggs by an order as compared to the tables of chemical composition; as a result, such an egg can ensure the intake from 5 up to 10 % of an adequate consumption of carotenoids [43]. Emulsification of carotenoids provides their desired concentration when the used doses are 20-30 % lower [54]. Diet supplemented with 2.5 % spirulina powder (*Arthrospira platensis*) or 30 mg/kg Lucanthine was equally effective in enhancing egg yolk colour [55]. Increasing doses of dietary lycopene (0; 420 and 840 mg/kg) and α-tocopherol (0; 84; 164; 200; 284 and 364 mg/kg) was accompanied by an increase in lycopene ($p < 0.05$) and vitamin E ($p < 0.05$) in the egg yolk, whereas lutein and zeaxanthin concentrations remained constant [56]. The amount of egg yolk lutein depends on its dietary intake according to a saturation curve pattern, that is, the egg pigment first increased from 0.3 to 1.5 mg/60 g with increasing dietary lutein up to 375 mg per 1 kg, and then remained unchanged regardless of further rise of its dietary dose [27].

Lutein is the main carotenoid which prevents macular degeneration during ageing. Lutein of fortified egg yolk is absorbed better compared to isolated lutein or lutein contained in plants [57]. Daily consumption for 4.5 weeks of 1.3 yolks of chicken eggs, which provides 0.38 mg of lutein and 0.28 mg of zeaxanthin, lead to increased blood concentration of these carotenoids by 28-50 and 114-142 %, respectively [43]. At the same time, the carotenoid intake due to the yolks was equivalent to consumption of 60 g of spinach dishes or 150 g of corn dishes. As lutein is a specific carotenoid which concentrated in the retina yellow spot, dietary products enriched with bioavailable lutein can be considered as a factor that reduces the risk of age-related macular degeneration [26, 58].

B vitamins. Dietary B_1 provides its increase in the egg by about 25 %, and vitamin B_2 supplements can lead to a 1.7-fold increase in B_2 level in eggs (see Fig., B, D).

Women of childbearing age with a non-optimal folate status have risks of children being born with neural tube defects, and the clear linkage between folate concentration, homocysteine in the blood, and risk of cardiovascular diseases is shown [59]. Natural folic acid (pteroyl-L-glutamine) is found only in trace amounts. The 50-80 % of natural forms of this vitamin in foods are polyglutamates, the 5,6,7,8-tetrahydrofolates (H4 folates). Bioavailability of natural folate is lower than that of folic acid. This difference is partly due to the fact that folic acid can be absorbed directly, while folates (mainly polyglutamates) are preliminarily hydrolyzed by disconjugase to monoglutamyl phosphate. Due to its high stability, folic acid is the only form of folate, which is added directly to food products and is part of multivitamin mixtures for animals. Among the opponents of the technological vitaminization method of food, there is a view that enrichment with folic acid leads to an increase in the amount of unmetabolized folic acid in the human body. As mentioned above, biofortification, in the process of which biotransformation of folic acid in the chicken organism proceeds, rejects this argument.

The egg folate content dependence on folic acid addition to the barley diet (0-128 mg/kg during 21 days) had the shape of the saturation curve. The folate content reached maximum 32.8-42.2 μg per egg, when adding synthetic folic acid up to 2 μg/kg feed, and further remained constant [24]. According to other data, enrichment of eggs with folate up to 41.0±0.7 μg occurred at a dose of 4 mg folic acid per 1 kg of barley-based feed, and this value remained stable during 28 days of storage at 4 °C [60]. A twofold increase in the folate content per egg melange occurred when 3.5 mg of folic acid per 1 kg of feed was added [61]. Supplementation of hen diet with folic acid (from 0 to 32 mg/kg) for 12 weeks increased folate amount in the egg to 75 μg, or 2.3-fold [25]. In the egg, folate is in the form of 5-methyltetrahydrofolate and only 10 % is folic acid. In vivo experiments on rats showed that the folate of folate-enriched eggs of hens, which were fed with its dietary form, was well absorbed in the body [62]. The excess of folic acid in the corn- and soybean-based diets (50 mg per 1 kg) was accompanied by a decrease in feed intake by hens and a reduced hatchability [63].

A relationship between the vitamin content in the hen diet and in the laid eggs [64] found in all cases (see Fig.) shows that the amount of egg vitamins can not increase unlimitedly, but tends to a certain maximum value, when a further increase of dietary vitamins will not lead to an additional increase in their content in eggs (see Fig.). That is, the eggs are saturated with these micronutrients [16, 17]. Excessive dietary vitamins can decrease productive performance of laying hens [63].

It is obvious, that to achieve the maximum amount of each vitamin, special researches are required to determine its optimal content in the diet. In this case, excessive consumption of some vitamins can disrupt the balance of others in the egg. Thus, excessively high vitamin A content in the diet (400,000 IU/kg) led to a decrease in vitamin E and carotenoids in the yolk [19, 65]. Significant decrease of the vitamin E concentration in the yolk was the result of simultaneous enrichment of hens' diet with vitamin E and β-carotene (200 mg/kg feed) [18]. An increase of the canthaxanthin level in the diet from 12 up to 24 mg per 1 kg caused an increase in γ-tocopherol amount in the egg yolk [66]. An increased amount of all dietary vitamins led to a simultaneous increase of folate, biotin and pantothenate, vitamins A, E, B_1, B_{12}, D, and $25OHD_3$ ($p < 0.05$) in eggs [67]. Simultaneous enrichment of chicken eggs with vitamins D, K and iron showed that the addition of 12000 IU of vitamin D_3 and 7.5 mg of vitamin K per 1 kg of feed for 20 days elevated the levels of these vitamins 4.6 and 4.8 times, respectively [68].

Given the functional links between vitamin in the body, the simultaneous intake of vitamins is more effective for improving body state [69], so the maximum egg enrichment to an amount that meets the criteria for enriched food products [70] seems very prospective.

Thus, through optimal vitamin enrichment of the poultry feed, eggs with a maximum content of vitamins and carotenoids can be obtained, which significantly increases their nutritional value. One egg can provide up to 40-50 % of the recommended daily intake of vitamins D, B_{12}, K, pantothenic acid, 30 % of vitamin E, 20 % of folate, about 10 % of vitamins A and B_2, and up to 30 % of adequate amount of lutein consumption. In this case, enrichment with vitamins meets the criteria for food production, i.e. from 15 up to 50 % in a portion. Biofortification of hen eggs with vitamins is one of the effective strategies providing population with vitamins.

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