11 Simultaneous Enrichment of Eggs With PUFAs and Antioxidants

Prospects and Limitations

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Abstract

The recent data indicate that a designer egg enriched in vitamin E, lutein, DHA, and Se can be not only a good nutritional product but also a good vector for the delivery of four essential nutrients vital for human health. A crucial feature of these designer eggs is the synergistic combination of n-3 fatty acids with major antioxidants, vitamin E, lutein and Se, as an important approach to the improvement of the human diet. These eggs will not be able to replace vegetable and fruits as a major source of natural antioxidants and fish products as a source of DHA but can substantially improve the diet, especially in countries like Scotland, significantly contributing to the recommended daily intake of vitamin E, lutein, DHA, and Se. Commercially, it is possible to produce designer eggs enriched with four nutrients or with three, two or one nutrient(s) depending on the consumer demand. As a result, price for the production of such eggs could substantially vary. Therefore the way of egg to the functional food category started successfully and now it is consumer education which is needed to fulfill the idea of using eggs as functional food.

Key Words: Eggs; vitamin E; carotenoids; selenium; antioxidants; omega-3 fatty acids.

1. INTRODUCTION

Recent achievements in biochemistry and molecular biology, together with epidemiological data have changed our thinking about food. It has become increasingly clear that our diet plays a pivotal role in maintenance of our health and that an unbalanced diet can cause serious health-related problems. It seems likely that ω-3 fatty acids and antioxidants are among the major regulators of many physiological processes and therefore a balance between ω-3/ω-6 PUFAs and antioxidants and prooxidants in the diet, gastrointestinal tract, plasma and tissues is an important determinant of the state of our health.

2. DESIGNER EGGS AS A WAY TO IMPROVE HUMAN DIET

For the last few years designer egg production has made a substantial progress in many countries of the world. In particular, ω-3 enriched eggs can be found on supermarket shelves in Europe, The United States, Australia, Malaysia, and Thailand, among others. However, the ω-3 eggs comprise only the first step in the manipulation of egg composition. In particular, natural antioxidants have attracted substantial attention in

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relation to egg quality *(1)*. When considering possibilities of egg enrichment with antioxidants it is necessary to take into account the following:

- Efficiency of nutrient transfer from the feed to the egg. For example, vitamin E and lutein are effectively transferred to the egg, efficiency of vitamin A transfer is much lower and ascorbic acid is not accumulated in the egg at all *(1)*. It is possible to double folic acid concentration in the egg (achieving about 10% RDA), but it seems that further enrichment is limited *(2)*. There are no data available on the flavonoid transfer to the egg.
- Form of nutrient in the diet. Inorganic selenium (Se) in the form of selenite or selenate is characterized by a relatively low efficiency of transfer to the egg. However, organic Se in the form of Se-enriched yeast (Sel-Plex) is transferred to the egg much more effectively. This makes it viable to enrich eggs with Se *(3)*. Either the oil or dry forms of vitamin E are suitable for inclusion into the diet.
- Availability of commercial sources of effective feed forms of antioxidants. Vitamin E (DSM/ROCHE, BASF, etc), organic Se (Alltech, Inc), lutein (Kemin) are commercially available.
- Possible toxic effects of nutrients for the laying hens. For example, vitamin A in high doses in the chicken diet can be detrimental for their health *(4)*, it is not effectively transferred to the egg yolk *(5)* and the elevations that are achieved *(4)* are still far away from the daily requirement in this vitamin. There is a suggestion that vitamin D enriched eggs could be useful for diet improvement for senior citizens *(6)*. In fact, it is possible to commercially produce eggs containing about 40 to 50% RDA in this vitamin *(7)*. However, as with vitamin A, an excess of vitamin D in the hens' diet could be toxic for the bird *(8,9)*. Very high Se doses in the form of sodium selenite could also be detrimental for chicken health and therefore there are legal limits on amounts of Se which can be included into poultry diets. In EU countries, the maximum amount of Se permitted in poultry diets is 0.5 ppm, whereas in the US the permitted level is 0.3 ppm *(3)*. However it is interesting to note that legal limits of Se supplementation are set not because of its toxicity, but because Se is considered as an environmental pollutant.
- Amount of nutrient delivered with an egg in comparison with RDA. If enriched eggs are going to make a significant contribution to human health then arguably they should be capable of delivering an amount of a nutrient comparable with RDA. Our data *(1,3)* showed that with a single egg it is possible to deliver all the daily requirement of vitamin E (15 mg) and 50% of the RDA for Se (30 μg). This became an important marketing tool: "add one egg to the diet and the requirement will be met."
- Established health-promoting properties of nutrients and their shortage in a modern diet. The justification for inclusion of vitamin E into the egg is clear. It is an important component of the antioxidant defences, in many cases diets are deficient in this antioxidant and consumption of high doses of vitamin E (higher than RDA) is beneficial. The same is true for lutein, a carotenoid that has well-established health-promoting properties *(1)* but is often deficient in the modern diet. Furthermore, the health-promoting properties of Se for maintaining health of general public are increasingly being recognized *(3)*. Thus, in most of developed countries Se deficiency is a common feature, while on the other hand, the cancer-preventive properties of Se, and the importance of maintaining the optimal Se status for promoting health, are emerging from recent research *(3)*.
- Possible interactions with assimilation of other nutrients from the egg. When an egg is enriched simultaneously with vitamin E and/or lutein, the lipids of egg yolk could

help antioxidant assimilation. In fact, the amount of lipids in the egg yolk (about 6 g) and their composition (i.e., saturated, monounsaturated and polyunsaturated fatty acids) could provide an ideal milieu for vitamin E and/or lutein absorption by the human intestine. On the other hand, vitamin E, lutein and Se can prevent ω-3 peroxidation during absorption.

- Stability during egg cooking. Vitamin E, lutein and Se are quite stable during egg boiling or frying.
- Effect on appearance and taste. Vitamin E, carotenoids and Se do not affect the organoleptic characteristics of an egg other than helping to prevent the development of a "fishy taint" in ω-3 eggs. Egg enrichment with lutein could be beneficial (in some countries) in terms of consumer preference of deep coloured egg yolk.
- Health claim regulations. Health claim regulations differ substantially from country to country. For example, in Malaysia there are no restrictions on health claims and such claims as "delays the onset of aging" or "increases fertility" can be found on egg box. However in other countries, such as north America and those in the European Community health claims need to be substantiated.

There are also important points in relation to the choice of products for antioxidant enrichment (Table 1) and eggs seem to be an ideal product for this purpose.

3. DIFFERENTIAL RESPONSIVENESS OF EGG COMPONENTS TO DIETARY MANIPULATION

As mentioned above, the levels of some micronutrients of the yolk can easily be increased by supplementation of the diet of the hen, whereas other components are more resistant to change. The explanation for these differences lies in the specific mechanisms whereby the various yolk components are incorporated into the oocyte during vitellogenesis. The lipids of the yolk derive from the uptake of VLDL-type lipoproteins from the hen's plasma. The lipid-soluble antioxidants, vitamin E and carotenoids, are dissolved in the lipid matrix of the lipoproteins. Because of the vast molecular excess of lipid, these lipoproteins can easily accommodate the increased amounts of vitamin E and carotenoids that are provided by dietary supplementation. Thus, the levels of these antioxidants in the egg increase linearly with their concentrations in the parental diet. There are, however, great variations in the efficiency of incorporation of the different types of carotenoids. Lutein, for example, is incorporated into the egg with high efficiency. On the other hand, β-carotene is poorly incorporated into the egg of the chicken, probably because of its conversion to vitamin A in the tissues of the hen. There are species differences here since eggs of many species of wild birds actually contain large amounts of β-carotene. These concepts have been discussed by Surai *(1)*.

Water-soluble vitamins such as thiamin (vitamin B_1), riboflavin (vitamin B_2) and biotin are transported in the hen's plasma, each as a complex with its specific binding protein. These vitamins, together with their binding proteins, are taken up by the oocyte from the plasma and are delivered into the aqueous fraction of the yolk. As discussed by White *(10)*, the levels of these vitamins in the yolk do not increase linearly with dietary supplementation. After an initial increase, the binding proteins in the plasma become saturated with their respective vitamins and so the concentrations of the vitamins in yolk rapidly reach a plateau. Thus, the capacity for enrichment of yolk with these vitamins is restricted by the availability of their binding proteins.

Table 1
Some Characteristics of Food Choice for Se-Enrichment*

The food should be	*Comments*
A part of traditional meals for the population	It would be counter-productive to attempt a change in culturally-based food habits by introducing a new type of food. Emphasis should be given to the possibilities of changing composition of existing foods such as by selenium enrichment.
Consumed regularly in a moderate amount	Because the objective is to deliver the amount of selenium needed to meet RDA it is necessary to choose food which is consumed regularly in moderate amount. Over-supplementation is unnecessary and undesirable.
Consumed by the majority of the population	This is particularly important given that immune function is more likely to be compromised in groups such as children and the elderly.
Affordable	Affordability of food would play an important role in the consumer choice.
Enriched with other health-promoting nutrients that are in short supply in the same population	Examples of minerals critical to health that are frequently deficient include iron and iodine. Vitamin E and lutein are also in short supply in the human diet. This can give a greater improvement in the diet.
Supplying a meaningful amount of the nutrient (e.g., at least 50% RDA)	This is an important point that distinguishes true functional foods from products that include 'tag-dressing' amounts of nutrients for advertising purposes.

*Adapted from ref. *3*.

Although vitamins A and D are lipid-soluble vitamins, they are not located in the lipoprotein fraction of the yolk. Instead, they are partitioned into the aqueous fraction, complexed to their respective binding proteins. Thus, the ability to enhance the levels of vitamins A and D in yolk is subject to the same limitations as described for the water-soluble vitamins. Some elevations of the yolk content of vitamins A and D are, nevertheless, possible because their plasma binding proteins are present in some excess.

Saturated fatty acids, as a proportion of total yolk fatty acids, are resistant to dietary manipulation. Likewise, only modest changes in the proportion of oleic acid (18:1 ω-9) in yolk lipid are achieved when the diet of the hen is supplemented with this fatty acid. Birds tend to maintain the proportions of saturated and monounsaturated fatty acids in yolk within fairly narrow margins, irrespective of diet composition. These constraints result largely from positional specificities. For example, saturated fatty acids are preferred at the sn-1 positions of both the triacylglycerol and phospholipid of the yolk. On the other hand, the PUFA profile of yolk is very responsive to changes in the pattern of dietary PUFA. Thus, supplementation of the hen with oils that are rich in either 18:2 ω-3, 18:3 ω-3 or DHA readily elevates the proportions of these respective PUFA in the yolk. Inclusion of increasing amounts of DHA-rich fish oil in the hen's diet initially produces a linear increase in the representation of DHA in the yolk lipids. In fact, the relationship between dietary DHA and yolk DHA can be described with precision by linear regression equations *(11)*.

There are, however, limits to the incorporation of DHA into yolk lipids. At high levels of supplementation, linearity of the response is lost and the proportion of DHA in yolk approaches a plateau *(12)*. First, this is because the main lipid fraction of yolk, triacylglycerol, normally contains only a trace of DHA, and this proportion is hardly changed by supplementation. The yolk phospholipid fraction is the main site of DHA incorporation, and so this fraction, accounting for only about one-quarter of the total yolk lipid, has to accommodate essentially all of the increase in yolk DHA content that is induced by supplementation. Second, the DHA of yolk phospholipid is located exclusively at the sn-2 position in the molecule. Thus, DHA has access to only half of the potential incorporation sites in yolk phospholipid. Third, the acyl substrate specificity of the acyltransferases that incorporate fatty acids into the sn-2 position of yolk phospholipid mitigates against the unlimited incorporation of DHA at this site. Supplementation of the hen with fish oil enforces the replacement of arachidonic acid (20:4 ω-6) by DHA at the sn-2 position of yolk phospholipid. However, this replacement is never total, as some 20:4 ω-6 is always retained at this position. Also, DHA has to compete with 18:2 ω-6 for esterification into yolk phospholipid. Obviously, for these reasons, it is never possible to produce a yolk in which DHA accounts for a major proportion of the total fatty acids. Nevertheless, by appropriate supplementation, it is possible to raise the level of DHA in yolk lipid to values approaching 4% of the total yolk fatty acids. This represents a four- to five-fold increase over the control value, and is only slightly lower than the proportion of DHA that is achieved in yolks of piscivorous birds such as penguins.

For the purpose of producing eggs that are enriched in health-promoting nutrients, it is extremely fortuitous that ω-3 PUFA, vitamin E and carotenoids all fall into the class of egg constituents that can readily be elevated by dietary means. It is equally providential that yolk Se, uniquely among trace elements, is highly responsive to dietary supplementation, particularly with an organic source of this antioxidant. Again, this reflects the mechanisms of nutrient uptake into the yolk during vitellogenesis, because Se is incorporated into yolk proteins as seleno-amino acids, whereas other trace elements must compete for binding sites on yolk phosvitin. The possibility of producing eggs enriched with a health-promoting combination of ω-3 PUFA and a range of key antioxidants is, therefore, greatly favored by the particular range of uptake mechanisms that have evolved to provision the egg with a wide range of nutrients.

4. SUPER-EGG DEVELOPMENT AND EVALUATION

Recent scientific evidence reinforces the importance of eggs as a healthy food choice. Our attention has been attracted by eggs as a most convenient delivery system for nutrients of our choice: vitamin E (the most abundant lipid-soluble antioxidant), lutein (one of the most important plant carotenoid pigments), Se (a trace element), and DHA (an important long chain PUFA belonging to the ω-3 family).

Based on the results of analyses of eggs obtained from certain wild and free-range birds *(13–15)*, which were characterized by very high concentrations of ω-3 fatty acids, vitamin E, lutein, and Se, it was decided to produce an egg that had enhanced levels of these components, a so-called "super egg." Our idea was to produce an egg containing vitamin E, lutein, DHA, and Se in amounts comparable with the daily requirements of these nutrients in a palatable and visually acceptable form. The main concept was "healthy eggs from healthy birds" because all these four nutrients are as important for the hen's health as for human health.

Table 2
Major Nutrients in a Super Egg

Nutrient in the egg	*Amount (mg)*	*% Recommended Dietary allowances*	*Similar amount provided by*
Vitamin E	19.3	150	100 g corn oil
			150 g margarine
			300 g peanuts
			1 kg butter
			10 kg meat
Lutein	1.91	RDA not known	50 g celery
			100 g green peas
			200 g asparagus
			200 g green pepper
			200 g yellow pepper
Selenium	0.032	50	100 g wheat bread
			150 g brown bread
			500 g meat
			1 kg vegetables
DHA	209	100	49 g sardine
			165 g Atlantic cod
			170 g haddock
			180 g carp

Adapted from refs. *55,56*.

By manipulating the feed of laying hens it was possible to enhance the levels of Se, vitamin E, lutein, and DHA by 7.7, 26.8, 15.9, and 6.4-fold respectively. A single designer egg contained 50% of the RDA of Se, 100% of the RDA of ω-3 HUFA, and 150% of the RDA of vitamin E. It also supplied 1.91 mg lutein (no reference nutrient intake has yet been established) (Table 2).

We conducted a human trial where 44 healthy adult volunteers (24 men and 20 women: minimum, mean and maximum ages respectively were 26, 41.1 ± 1.5, and 59 yr) were recruited and participated in an ethically-controlled trial. The volunteers did not use vitamin E, carotenoid, Se or fish oil supplements and were not using medically prescribed diets or slimming regimes. Subjects were stratified by age and sex and then randomly allocated to either a designer or a commercial table egg per day in a double-blind trial.

Our results indicate that vitamin E is effectively transferred into the egg *(16)* and its concentration in the egg yolk is a reflection of the dietary supplementation. Very high levels of vitamin E can be reached in the egg yolk *(1)* without compromising the productive characteristics of the hen *(17)*. We have chosen an amount of vitamin E/egg (about 20 mg) which provides about 150% RDA and the results of the human trial indicate that the consumption of designer eggs significantly increased the plasma vitamin E concentration over that of the control group and was effective in all treated subjects *(18)* (Table 3). A similar response in plasma vitamin E was found after a 10-wk consumption of antioxidant-enriched margarine providing 31 mg vitamin E/d *(19)*. Plasma γ-tocopherol and vitamin A concentrations were unaffected by treatment. This fact is

Table 3

Initial and Final Plasma Antioxidant Concentrations of the Control and Experimental*Groups (μmol/L)

	Control		*Experimental*	
Parameter	*Before*	*After*	*Before*	*After*
α-tocopherol	25.98	24.95	25.63	30.47
γ-tocopherol	2.10	2.20	2.02	1.98
Lutein	0.21	0.21	0.24	0.45
Vitamin A	2.31	2.17	2.13	2.15

Adapted from ref. *36*.

especially important because the consumption of high levels of ω-3 fatty acids in the form of fish oil by young women was associated with a decrease in vitamin E in plasma and a significant increase in plasma lipid peroxide *(20)*. A similar decrease in vitamin E concentration in plasma was reported for healthy male subjects consuming a daily fish oil supplement *(21)*. Therefore, combination of ω-3 fatty acids with natural antioxidants is an important strategy in human nutrition.

In our human trial *(18)* lutein concentration in plasma significantly ($p < 0.001$) increased (Table 3) in subjects consuming the designer eggs. The increased concentrations of vitamin E and lutein would significantly increase the antioxidant potential of the plasma and help to protect PUFs from peroxidation. Taking into account that vitamin E in the egg yolk is found in the easily digestible α-tocopherol form *(16)* and the highly positive response to vitamin E and lutein supplementation in this study, it seems likely that the bioavailability of these nutrients from cooked egg yolk is quite high. For example, lutein concentration in the plasma increased two-fold (similar to our case) after consumption of a more than five times greater amount of lutein (11.3 mg) from spinach powder *(21)*, but the experiment was shorter (2 wk) compared with 8 wk of egg consumption in this study. Egg yolk contains about 6 g of lipids including saturated, mono- and polyunsaturated fatty acids *(13)* and as mentioned above may be ideal for providing the necessary amount of lipids for mixed micelle formation in the lumen of the human intestine, an important step in lutein and vitamin E absorption *(16,22)*. It has been suggested that increased intake of vitamin E through selection of foods and daily consumption of between five and eight servings of fruit and vegetables rich in carotenoids may reduce risk for cardiovascular disease and improve immune function in later life *(23)*.

In our experiment, when volunteers had adequate Se levels in the diet, consumption of the designer eggs enriched with Se did not affect Se concentration in plasma *(18)*. Se availability from eggs for human needs further elucidation. Possibly, the effect of different dietary sources of Se on its concentration in plasma depends on the Se status of the human. For example, in the case of low-Se status, the consumption of eggs enriched with Se for 3 mo significantly increased serum and hair Se levels *(24)*. Similarly, in Chinese children from the area endemic for Keshan disease (a cardiomyopathy that is closely associated with very low dietary intakes of selenium), Se content in hair increased due to consumption of Se-enriched eggs for 3 yr *(25)*.

Our results showed that 8 wk of intake of control or designer eggs did not alter either total lipid concentration or the proportions of the lipid classes *(18)*. There was no significant effect of egg consumption on blood pressure, or on total and HDL-cholesterol in the blood. The consumption of DHA-enriched eggs resulted specifically in significantly higher proportions of DHA in each of the human plasma lipid fractions, with no significant changes in any of the other fatty acid components. In plasma phospholipids, DHA was increased 1.3-fold by the designer egg consumption. Increases in the proportion of DHA in the other fractions of plasma lipids ranged from 1.4-fold in cholesteryl ester to 2.3-fold in the plasma triacylglycerol and 1.6-fold in free fatty acid. These increases in plasma DHA affected all treated subjects with the exception of one subject who had had the highest baseline value. It may be suggested that the enhancement of plasma DHA concentrations by eating designer eggs could result in beneficial effects on various health-related parameters such as cardiovascular function, inflammation and immunocompetence *(26–28)*.

Our results indicate that two major antioxidant constituents of the egg, vitamin E and lutein, are stable when designer eggs are boiled *(18)*. In this experiment, a combination of high levels of two antioxidants vitamin E and lutein in the designer eggs significantly decreased malondialdehyde production during Fe-stimulated lipid peroxidation, in spite of the high content of the highly unsaturated DHA in the egg. Similarly, egg enrichment by vitamin E and carotenoids decreased cholesterol oxidation in egg lipids exposed to nitrogen oxide *(29)* or during egg powder preparation *(30)*. Vitamin E enrichment of the egg yolk protects carotenoids from oxidation as well *(30)*. Thus, it seems likely that the combination of two antioxidants, namely vitamin E and lutein, accumulated in egg yolk, may improve the storability of the designer eggs compared with normal table eggs, even in the presence of enhanced levels of DHA.

The major advantages of the combination of DHA and antioxidants in the egg yolk are:

- Vitamin E, lutein and Se protect DHA from oxidation during absorption and metabolism in the hen, preventing any "fishy" taste formation in the egg.
- Egg yolk lipids are necessary for the efficient absorption of vitamin E and lutein in human intestine (the 6 g of lipids in egg yolk is ideal for efficient absorption of vitamin E and lutein in the human intestine).
- Lutein interacts with vitamin E and phospholipids, increasing the yolk's anti-oxidant potential and improving egg storability.
- Se, as an integral part of the antioxidant enzyme glutathione peroxidase, protects intestinal membranes against lipid peroxidation during DHA digestion.

These eggs deliver key elements in the diets of pregnant women, the elderly, and young children. The inclusion of such eggs in different processed foods (e.g., mayonnaise and cakes) will increase their nutritional value. The eggs will be of great importance to people living in polluted areas (e.g., Chernobyl, Ukraine) and in areas with very low temperatures (e.g., Polar expeditions), and extreme conditions (e.g., submarine teams).

5. SE-ENRICHED EGGS AS A MODEL FOR FURTHER DEVELOPMENT OF DESIGNER EGGS

Se is a key component of a number of functional selenoproteins required for normal health. It is provided in the Western European diet mainly from bread and cereals, fish, poultry, and meat *(31)*. In most diets in the United States, the main food sources of Se are cereals, meats, and fish *(32)*. Selenomethionine represents the major natural form of selenium in feed and food ingredients. Se enters the food chain through incorporation into vegetable proteins as the amino acids selenomethionine and selenocysteine. The British government's defined reference nutrient intake is 75 μg/d for men and 60 μg/d for women *(33)*. USA RDI for Se are similar, at 55 μg/d for men and women. An intake of 40 μg/d was suggested as the minimum Se amount required for humans *(34)*. A great body of evidence indicates that European intakes of Se are falling. For example, in 1978 Se intake in Britain was 60 μg/d, 7 yr later it was only 43 μg/d, and in 1990 fell to 30 μg/d *(3)*. Even in 1997, the average reported Se intake was only 43 μg/d *(35)*. Dietary intakes of Se in other countries vary considerably but in many of them intake is still lower than the RDI (Table 4). Reilly *(31)* described more than 40 human diseases and conditions associated with Se deficiency.

Because the Se content in plant-based food depends on its availability from the soil, the level of this element in human foods and animal feeds varies among regions. In general, eggs and meat are considered to be good sources of Se in the human diet. When considering ways to improve human Se intake, there are several potential options. These include:

- Direct supplementation.
- Soil fertilization.
- Supplementation of food staples such as flour.
- Production of Se-enriched functional foods.

It seems likely that the fourth strategy, to produce "functional foods" enriched with Se, deserves more attention *(1,3,6)*. Indeed the production of Se-fortified eggs is extremely simple: when Se supplementation of the diet is at a level of 0.4 mg/kg diet in the form of Se-enriched yeast (Sel-Plex), an egg would contain approximately 30 μg of Se which is about 50% of the daily requirement. Se-enriched eggs could easily solve a problem of Se deficiency in countries such as Scotland. This valuable option awaits response from the food industry. However a lack of general knowledge in the field of natural antioxidants as well as in functional food potential benefits is the major limiting factor for wide use of such eggs by consumers.

It seems that Se in eggs is highly available for absorption. For example, a recent clinical trial conducted in the Ukraine showed that consumption of two Se-enriched eggs/d for 8 wk significantly increased the Se level of the plasma of volunteers *(37)*. In fact 60 volunteers (30 in control and 30 in experimental group) successfully finished the trial. Eggs consumed in the control group contained 7–9 μg Se/egg and experimental eggs were enriched with Se (28–32 μg Se/egg, levels similar to those found in Columbus eggs). Blood was collected before the beginning and at the end of experimental period and Se was determined in plasma by hydride generation atomic absorption spectrometry with fluorometric detection. The level of Se in plasma of volunteers living in the Kiev area of Ukraine (0.055–0.081 mg/mL) was on the low side of the physiological range and was somehow lower than we reported earlier in volunteers in Scotland *(18)*. Consumption of control eggs for 8 wk only slightly increased Se in plasma (0.075–0.085 mg/mL). In contrast, consumption of two Se-enriched eggs daily, which together delivered the daily requirement of between 55 and 65 μg Se, for 8 wk was associated with a significant increase in Se concentration in plasma. Plasma Se reached

Table 4
Low Daily Selenium Intakes in Selected Countries (µg/d)

Country	*µg/d*	*Year reported*
China, Keshan disease area	2–36	1985
China, Keshan disease area	7–11	2001
New Zealand, low-Se area	11	1984
Saudi Arabia	15	1997
Poland	11–40	2000, 2003
UK	12–43	1192, 1195, 1197, 1198, 2003
New Guinea	20	1992
Czech republic	15–50	2003
Nepal	23	1988
Finland before selenium fertilization	26	1987, 1984, 1985
India,vegan low income	27	1997
Egypt	29	1972, 1996
Serbia	30	2001
Slovenia	30	1998
China	26.0–37.2	2000
Croatia	27.3–33.9	1998, 2000
Slovakia	27–38.2	1996, 1998
Belgium	28.4–61.1	1989, 1994
Brazil	28.4–37.0	2004
New Zealand	29–38	1999, 2001, 2004
Sweden	29–44	1991, 2000, 2003
France	29–48	1994, 1994
Turkey	30–36.5	1996, 1997, 2004
UK, 1994	32	1997
UK, 1995	33	1997
England	35	2000
Spain	35	1996
Germany	35–48	1989, 2000
Portugal	37	1990
Denmark	38–47	2000
Italy	43	1985
UK, 1985	43	1997
India, conventional diet	48	1997
Austria	48	2001
Ireland	50	2002
UK, 1974	60	1997

*Se requirement is 55 µg/d (US) and 60 for women and 75 µg/d for men (UK).
Adapted from ref. *3*.

levels of between 0.09 and 0.14 µg/mL, indicating a much improved Se status of the volunteers *(37)*. This is the first clinical trial to prove that Se-enriched eggs could be used as an important vector to improve Se status in countries with low-Se consumption like Scotland or Ukraine.

Table 5
Vitamin E and Carotenoids in Egg Yolk of Birds (μg/g)

Species	*Vitamin E*	*Species*	*Carotenoids*
Wild birds			
Pelican	299.2	Pelican	294.0
Cormorant	150.9	Cormorant	115.7
Lark	156.6	Lark	169.2
Bluebird	206.0	Bluebird	122.3
Blackbird	185.1	Blackbird	185.3
Cowbird	168.7	Coot	131.0
Gannet	216.8	Northern Flicker	107.9
Grebe	228.0	American Kestrel	111.2
Mallard	94.3	Mallard	61.3
Free-range birds			
Free range guinea fowl	31.1	Free range guinea fowl	79.2
Free living pheasant	77.1	Free living pheasant	72.6
Free range chicken	66.3	Free range chicken	75.2
Commercial birds			
Chicken, 10 ppm	33.2	Commercial chicken, wheat-based diet	15.1
Chicken, 100 ppm	205.6	Commercial chicken, maize-based diet	30.2
Chicken, designer diet	1090.5	Chicken, designer diet	106.2

6. ANTIOXIDANTS IN EGGS FROM WILD BIRDS

It seems likely that low Se, vitamin and carotenoid levels in commercially produced table eggs is a reflection of the poultry diets used. As can be seen from data presented in Table 5, carotenoid and vitamin E concentrations in egg yolk from variety of wild species are quite high and in many cases exceed those in commercial table eggs. Furthermore, decreased Se levels in feeds and foods in many cases reflect consequences of our agricultural practices. Therefore, eggs or meat produced by free-range poultry/animals fed on natural feed sources grown on well-balanced soils 100 to 200 yr ago would contain much higher Se concentration than we currently have in many European and Asian countries. Again, by supplementing animal diets with natural organic sources of Se, we are returning back to nature. Our recent data on the Se profile of eggs from various avian species in wild *(15)* confirmed this idea: Se concentration in eggs of 14 avian species in the wild was found to be much higher than that in eggs that derive from commercial poultry production. Our results imply that the Se requirement for birds breeding in captivity will vary among species. Appropriate guidelines could be developed by considering the yolk Se concentration displayed by free-living counterparts of a species. The Se level in the chicken eggs even after organic Se supplementation *(36)* only raised the yolk Se level into the lower end of the range achieved by avian species in the wild, suggesting there may be scope for much higher levels of supplementation for poultry. It seems likely that the Se level which is considered to be the norm for table eggs is too low to be physiological and this should be studied more in detail in the future. Similar evidence of high-Se concentrations in wild water birds was related to eggs of little egrets, black-crowned night herons, and bridled terns from coastal areas of Hong Kong *(38).* In tissues of the seabirds from the Barents Sea *(39)*, from Alaska and arctic Russia *(40)*, as well as in bald eagles from Adak Island Alaska *(41)* selenium levels were also several-fold higher in comparison to domestic chickens. Furthermore, high-Se concentrations were reported in eggs from the tree swallow bank swallow and house wren *(42).* Therefore, Se-enrichment of eggs, meat, and milk is simply the production of naturally designed food ingredients. Indeed, production and commercialization of such organic Se sources as selenized yeast (e.g., Sel-Plex™) opened a new era in Se supplementation of animals and gave a real chance for producers to meet growing requirements of consumers. What is more, production of these kind of animal-derived foodstuffs is arguably a more consumer-acceptable way to health promotion.

Indeed, it is possible to provide consumers with a range of animal-derived products with nutritionally modified composition in such a way that they can deliver substantial amount of health-promoting nutrients such as Se to improve general diet and help to maintain good health. Therefore, without changing the dietary habits and traditions of various populations it is possible to solve problems related to deficiency of various nutrients, in particular selenium. The consumer will go to the same supermarket to buy the same animal-derived products (e.g., egg, milk and meat), cook and consume them as usual. The only difference will be in the amount of specific nutrients delivered with such products.

7. ANTIOXIDANT-ω-3 ENRICHED EGGS AS FUNCTIONAL FOOD

The concept of healthy food additives arrived from Japan in the 1970s and the term "functional foods" appeared in 1984 *(43).* At this time, consumers began to view food from a radically different vantage point. This "changing face" of food led to the development of a new area in the food and nutrition sciences *(44).* The Food and Nutrition Board of the National Academy of Sciences defines a functional food as one that encompasses potentially healthy products providing health benefits beyond that of the traditional nutrients it contains *(45).* This is in agreement with the data of the 1998 US study from written questionnaires, completed by 2074 respondents indicating that most shoppers believe foods can offer benefits beyond basic nutrition to functional nutrition for disease prevention and health enhancement *(46).* However, a recent US survey reported that taste is the primary influence on food choice, followed by cost *(47).* Similarly, in a survey in Ireland, "quality/freshness" of food was the most frequently selected food choice factor (51%) followed by "taste" (43%), and "trying to eat a healthy diet" (36%) *(48).*

Today, functional foods have received substantial attention *(31,49)* and represent one of the fastest growing segments of the world food industry *(43).* For example, dairy products and other processed foods, including mayonnaise, margarine and dressings containing DHA *(50)*, as well as ω-3 enriched eggs *(1)*, are already on the market in different countries. Antioxidant-fortified margarine is shown to be effective in the delivery of vitamins E and C as well as α- and β-carotene to humans *(19).* In the US, annual sales of functional food products comprise around $50 billion *(43).* In total, functional food has a market share of around 2% in the US food market and is quickly growing *(51).*

There are three major reasons for the increased interest in functional foods *(45)*: (i) increased health care costs, (ii) recent legislation, and (iii) scientific discoveries.

- Recently, six major targets in relation to functional food science have been identified *(52)*: (i) gastrointestinal functions, (ii) redox and antioxidant systems, (iii) metabolism of the macronutrients, (iv) development in fetal and early life, (v) xenobiotic metabolism and its modulation, and (vi) mood and behaviour or cognition and physical performance.
- In the same review the author has stated that the "health benefit of a functional food will be limited if that food item is not normally part of the diet," Therefore functional foods must remain foods and they must achieve their effects in amounts normally consumed in a diet *(53)*. Eggs have not traditionally been regarded as a functional food, primarily because of concerns about their adverse effects on serum cholesterol levels *(44)*. However recent findings described in this volume indicating that there is little if any connection between dietary cholesterol and blood cholesterol levels, as well as between moderate egg consumption and heart disease, could help to change any bad image of eggs. In this respect, eggs enriched with selenium as well as with a combination of Se, ω-3 fatty acids, vitamin E, and lutein, ideally fit into the category of functional food, enabling substantial improvements in diet quality.

For example, designer eggs could contribute to several aforementioned categories: redox and antioxidant systems (an egg delivers three antioxidant components, including 150% RDA in vitamin E, 50% RDA in Se and a substantial amount of lutein); development in fetal and early life (an egg deliverers a minimal RDA for DHA which is an essential element of the baby brain development), and mood improvement (an egg deliveries 50% of RDA in Se which is considered as having an effect on human mood). Indeed, increased ω-3 PUFA levels in pork and chicken, with simultaneous Se and vitamin E enrichment could have multiple benefits. As in the case of designer eggs, meat enriched with ω-3 PUFA needs increased antioxidant protection. This protection could be provided by increased Se and vitamin E concentrations. In fact there are currently a number of vitamin E-enriched meat products on the market, including sausages and cooked ham *(54)*. It is necessary to underline that Se-enriched eggs, meat and milk could have a positive effect on gastrointestinal function. Lipid peroxidation in the gut is believed to be one of the major contributing factors in the development of various gastrointestinal disorders, which are associated with enterocyte damage, inflammation of the mucosa, and the development of malabsorption.

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