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NUTRITION

Selenium interactions with essential and toxic elements in egg yolk from commercial and fortified eggs

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Abstract

The main objective of this work was to evaluate the interaction between selenium concentration in both commercial and Se-enriched eggs and other essential/toxic elements (Ca, Mg, Fe, Zn, Pb, and Cd), taking into account a possible synergic action of iodine. Commercial eggs were purchased from several sale points or directly from the producers (farmyard eggs). Fortified eggs were obtained by supplementing chickenfeed for 6 weeks with Se as sodium selenite (1.0 μg/g Se) or Se plus iodine (1.0 μg/g Se + 3.7 μg/g I). Se in experimental egg yolks significantly increased over the basic value by 39% in the Se group and 61% in the Se + I group, suggesting that I addition may enhance Se absorption. Levels of Se in commercial yolks were identical in free-range, barn or battery eggs, but significantly lower in farmyard and higher in organic eggs where the Se content approximated that found in Se fortified eggs. A significant reduction in Cd was observed in Se + I treated yolks compared to both control and Se alone diet, thus suggesting a high sensitivity of Cd to the detoxifying effect of Se combined with I. Furthermore, Se + I supplementation was associated with a significant Zn reduction, a finding which needs clarification to avoid attempts to maximize one component affecting the levels of other essential elements.

Keywords: Selenium; Trace element interactions; Eggs; Functional food

Introduction

Selenium (Se) is an essential trace element for animals and humans. It is a component of several major metabolic pathways, the antioxidant defence systems and immune functions [1–3]. Chronic marginal Se deficiency may enhance susceptibility to viral infection, cancer, cardiovascular disease, thyroid dysfunction and various inflammatory conditions [4–7]. Diet is the main source of Se in the general population and evidence suggests that in some regions Se is declining in the food chain [8–11]. In Italy, for instance, a study on mineral intake of different population groups living in communities confirmed that Se can be below the recommended dietary allowances, particularly among the elderly [12]. As a consequence, strategies to increase Se intake have been implemented, such as Se fertilisation of crops and vegetables, human and animal supplementation and education to consume higher-Se foods. Another strategy is to modify food composition through so-called functional food [13–15].

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In addressing specific health concerns, particularly protection against chronic disease, eggs deserve special attention as they are an excellent dietary source of many essential (e.g., protein, choline) and non-essential (e.g., lutein/zeaxanthin) components which may promote optimal health [16]. In general, most trace minerals are deposited in the yolk, whereas their concentration in the white portion of the egg is limited [17–20]. Se exhibits a variable distribution in egg yolk and ovalbumen depending on the chemical form of Se fed to the hen. Organically bound and natural forms of Se, such as selenomethionine and selenized yeast, promote deposition into egg white. Conversely, inorganic forms such as selenite result in greater Se concentrations in egg yolk [17,21–24].

Egg's nutrient content can be modified by the feed given to chickens, thus eggs with reduced content of cholesterol and saturated fat and increased vitamin E and omega-3 fatty acids have been produced [25,26]. It is not surprising that by avoiding intake of tablets, Se-enriched eggs have been designed and introduced in various markets, but not in Italy [27–31]. Concomitant enrichment with iodine is also common in view of the well-known iodine deficiency in many countries worldwide where this new strategy could accompany existing salt iodization programmes.

Most literature reports on Se and I enriched eggs paid little attention to the possible interaction between these two compounds and other minerals, even though Se has been implicated in regulating or normalizing levels of other mineral nutrients at key sites in the body [32]. Various *in vivo* and *in vitro* studies reported that the absorption, distribution and retention of Zn, Fe, Cu were affected by either Se deficiency or supplementation, but results are controversial and need confirmation [33]. A role of Se alone or in combination with Zn in decreasing the toxicity of heavy metals such as cadmium and lead has been documented in vegetables enriched through the soil supplementation [34–38]. These interactions could be stressed in the presence of iodine, as a synergism between iodine and Se through the Se-dependent peroxidase enzymes involved in iodine oxidation has been suggested [39–42].

Considering that Se-enriched eggs are becoming popular, and eggs remain a staple food in the human diet throughout the world, this study selected eggs as a model to study the relationship between Se and other elements. Se was measured and its possible role in counteracting Cd and Pb was investigated in both experimental Se-enriched and Italian commercial products. In addition, interactions with Ca, Mg, Zn and Fe were analysed and a possible synergic action with iodine was evaluated in supplemented eggs.

Materials and methods

Commercial eggs

Commercially available, fresh, class A eggs, selected according to different production methods (organic, free-range, barn or battery), were purchased from several sale points by selecting different producers and packaging centres, or directly from the producers themselves on their own farms (farmyard eggs). A total of 33 samples were analysed, consisting of pooled samples from the same carton of six eggs each. For organic, free-range, barn and farmyard eggs, six samples each were taken, whereas for battery eggs nine samples were taken. Eggs from barn, battery and free range are characterized by different production systems regarding the type of housing, but identical diet consisting in industrial chickenfeed. In addition to living conditions suitable for laying hens, organic eggs differed in the type of diet which consists of organic certified cereals and is richer in essential vitamins, minerals and trace elements. The farmyard eggs selected for our study were from hens nourished only with wheat or corn, with no other addition.

Animals and experimental eggs

After a 25-day pre-experimental period to adapt themselves to the diet, temperature and humidity conditions, 54 18-week-old Warren laying hens with similar body weight (2.0 kg) and egg parameters, were divided into three groups of 18 birds each (six replicates of three hens each) and allocated in cages (three animals in each cage).

The composition of the complete basic diet was as follows: corn 63%, soybean 24.20%, $CaCO_3$ 8.50%, corn oil 1.90%, CaH_2PO_4 1.5%, NaCl 0.4%, methionine 0.1%, vitamin–mineral premix 0.4%. The mineral premix provided per kilogram of diet: 40 mg Fe; 4 mg Cu; 40 mg Zn; 48 mg Mn; 0.8 mg I; 0.40 mg Co; 0.16 mg Se, according to the maximum permissible values in the European Union (EU Council Directive, 2004) [43].

Throughout 6 weeks, animals received this complete isonitrogenous and isoenergetic diet: (C) control group fed with the complete diet in which Se measured was 0.4 μg/g; (Se) the Se group fed with the control diet plus 2.03 μg/g sodium selenite (reaching a final concentration of 1 μg/g Se); and (Se + I) the Se + I group fed with the control diet containing 2.03 μg/g sodium selenite plus 5.0 μg/g potassium iodide (reaching a final concentration of 1 μg/g Se and 3.7 μg/g I).

During the experiment feed and water were provided *ad libitum* and egg-laying rate and dietary intake were recorded.

Eggs were sampled during a 3-day period at 0 and at 42 days from the start of the experiment. Eggs collected from each cage unit were mixed, and six samples from each treatment were used to determine Se and other minerals (Ca, Mg, Fe, Zn, Pb and Cd).

Methods

Egg whites and yolks were separated and stored in a cold room at 2 °C until homogenization, then freeze-dried. All samples were digested in a H_2O_2/HNO_3 (1/3) mixture in a microwave-irradiated closed vessel system (Milestone, mod. Ethos) and subsequently brought up to 10 mL with deionized water. Se was determined by fluorometry according to a slightly modified Olson et al.'s [44] procedure as previously described [45]. Briefly, 5 mL of EDTA were added to the digested solution and the pH adjusted to 1.8; 5 mL of 0.1% 2,3-diaminonaphthalene (DAN) (Fluka) solution were then added in a dark room, and the solution was heated to 50 °C for 30 min. This allowed formation of the fluorescent naphtho-2-seleno-1,3-diazole (4,5-benzo-piazselenol) complex, which was extracted. Se was determined by fluoro-spectrophotometry (Perkin-Elmer model 204) equipped with a xenon power supply (Perkin-Elmer model 150). The fluorescence of the extracted complex was measured with the excitation wavelength set at 375 nm and emission wavelength at 520 nm. Blanks and Se standard solutions were included in each set of determinations.

Zinc, Fe, Mg and Ca were analysed by air-acetylene flame atomic absorption spectrometry (Perkin-Elmer A-analyst 200), whereas Pb and Cd were analysed by graphite-furnace atomic absorption spectrometry (Perkin-Elmer A-analyst 600 equipped with an auto-sampler AS 800). The Ca determinations were performed in 0.1% lanthanium solution to prevent phosphate interferences. Instrument calibration standard solutions were prepared from commercial materials. Analysis of the NIST Standard Reference Material (SRM, No. 8415 whole egg powder) gave values of 2.42 ± 0.21 mg/g Ca (vs. a certified value of 2.48 ± 0.19 mg/g), 310 ± 13 μg/g Mg (vs. a certified value of 305 ± 27 μg/g), 120 ± 11 μg/g Fe (vs. 112 ± 16), 68.6 ± 6.0 μg/g Zn (vs. 67.5 ± 7.6), 1.41 ± 0.04 μg/g Se (vs. 1.39 ± 0.17), 0.061 ± 0.006 μg/g Pb (vs. 0.061 ± 0.012), 0.005 ± 0.001 μg/g Cd (vs. 0.005). Mineral concentrations are expressed as dry weights (dw).

Statistical analyses were performed by SPSS/PC v.12.0. All data were expressed as mean values and standard deviations. Normality of data distribution was tested by the Kolmogorov–Smirnov test. Paired Student's *t*-test was applied to test differences between the beginning and end of each treatment. Pearson' correlations were performed to evaluate the relation between elements. Comparison between groups (experimental and/or commercial eggs) was made by one-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc *t*-test. Differences at $p < 0.05$ were considered significant.

Results

During the experimental trial, egg production (C = 46.3%, Se = 41.2%, Se + I = 49.7%) and feed intake (C = 119.1 g/d, Se = 121.1 g/d, Se + I = 112.9 g/d) did not differ between groups and no significant difference in hen weight or weight change was detected at the end of the trial.

The average Se concentration in egg whites at the end of each trial was low and did not change with treatment (control: 0.15 ± 0.02; Se diet: 0.15 ± 0.04; Se + I diet: 0.14 ± 0.01 μg/g dw, respectively), thus only yolks were considered for this study.

Fig. 1 shows yolk Se content before and after treatment. Se content before treatment did not differ in the three groups. After supplementation with Se, Se concentration was 1.5-fold higher than unsupplemented yolk, and the increase was statistically significant. Se in yolks from hens fed with Se + I was even higher, and differed significantly from both control and Se group. By comparing pre- and post-trial yolks, both supplemented groups revealed a significant Se increase over the respective pre-treatment value. As a mean, a 39% increment for Se group (1.60 vs. 1.15 μg/g dw, $p < 0.001$) and 61% for Se + I group (1.98 vs. 1.23 μg/g dw, $p < 0.001$) was observed.

Table 1 presents the element's content in the experimental yolks at the end of treatment. Se enrichment was associated with a Zn reduction which reached statistical significance in the Se + I group. This combined treatment was also associated with a non-significant reduction in Pb levels and a significant decrease in Cd content ($p < 0.05$). These results were confirmed by the existence of a significant negative correlation between Se and Zn ($r = -0.525$, $p < 0.001$) and Cd ($r = -0.458$, $p < 0.05$) in experimental yolk at the end of treatment, not found before treatment.

The levels of essential and toxic elements in commercial egg yolks did not differ according to the category, except for Se and Cd as shown in Table 2. Free-range, barn- and battery eggs had identical Se content, organic yolks the highest value and farmyard yolks the lowest, both significantly different from any other category ($p < 0.05$). Cd concentration was significantly lower in organic and farmyard yolks compared to the barn category. Furthermore, Pb content was significantly lower (0.16 vs. 0.28 μg/g dw, $p < 0.05$) in farmyard yolks compared with the other categories taken together. Cd was positively related to Pb in both experimental

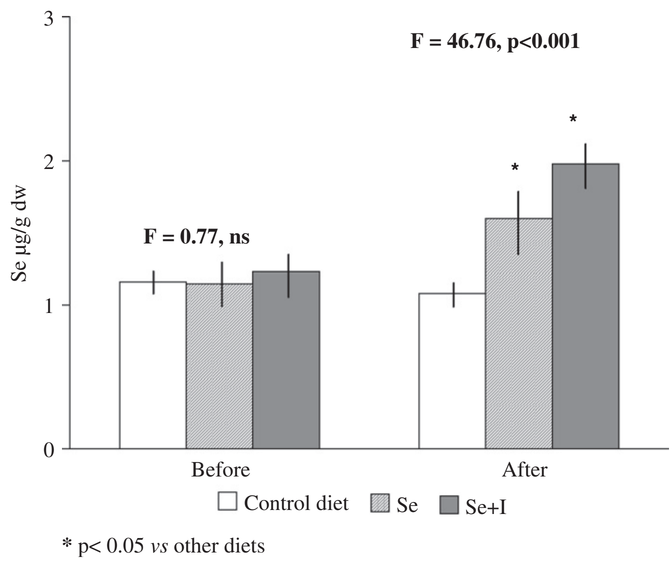

Fig. 1. Yolk Se content in both control and fortified eggs before and after treatment (mean ± SD). Eggs collected from each cage were mixed, and six samples from each treatment were analysed.

Table 1. Content of elements in experimental egg yolks at the end of treatment (mean ± SD)

Element	Group (n)		
	Control (6)	Se (6)	Se + I (6)
Ca (mg/g dw)	1.78 ± 0.17	1.77 ± 0.32	1.92 ± 0.32
Mg (µg/g dw)	189.5 ± 12.4	201.3 ± 29.2	188.7 ± 30.3
Fe (µg/g dw)	118.2 ± 9.1	112.8 ± 7.9	118.3 ± 8.1
Zn (µg/g dw)*	97.3 ± 21.3	86.3 ± 16.3	65.3 ± 15.3(a)
Pb (µg/g dw)	0.25 ± 0.19	0.39 ± 0.22	0.17 ± 0.10
Cd (ng/g dw)**	14.98 ± 6.73	15.88 ± 8.67	6.53 ± 1.00(b)

Eggs collected from each cage unit were mixed, and six samples from each treatment were used.
*$F = 6.42$, $p < 0.005$; (a)$p < 0.05$ vs. control.
**$F = 3.94$, $p < 0.05$; (b)$p < 0.05$ vs. other categories.

($r = +0.639$, $p < 0.005$) and commercial eggs ($r = +0.419$, $p < 0.02$). No other correlations between elements were found.

A direct comparison between experimental and market eggs in Se content expressed as total amount per yolk (assuming a mean yolk dw of 8.5 g) is presented in Fig. 2. Se content was significantly lower in market eggs compared to fortified products, except organic eggs which exhibited levels similar to those measured in Se-enriched eggs.

Discussion

Our study confirms that eggs containing 40–60% more Se in yolks can be produced by adding non-toxic levels of Se as sodium selenite in chicken feed. Our results also provide new information on the interaction between Se, I and other elements, an aspect scarcely evaluated in previous investigations [30]. First of all, we demonstrated that a synergism between Se and I exists. When iodine was added to the feed together with Se, the Se increment in yolks was significantly higher than in eggs treated with Se alone, suggesting that I enhances Se absorption and that the two elements act synergically. This is noteworthy as many fortified foods are enriched with both Se and I, and concerns about the potential for unacceptably high intakes need to be addressed, particularly in people consuming very large amounts of enriched food [46].

The second interesting result is the possible role of Se + I in detoxifying eggs towards Cd and Pb accumulation. Eggs treated with Se + I contained only 1.8 times more Se, but this increment was associated with a significant reduction in Cd and, to a lesser extent, Pb, despite very low concentrations of the two toxic elements in our study, and in eggs generally. Unfortunately, we did not measure iodine accumulation and we cannot clarify whether the effect is due to the higher-Se accumulation and/or to iodine. However, our results are in line with many studies supporting the protective effect of Se against Cd and other heavy metals [34,37,47,48]. A significant decrease of Cd was observed in both kidney and liver of mice acutely exposed to this metal after an 8-week daily Se supplementation [49].

Among the negative effects of supplementation, the Se + I diet was associated with a significant reduction in Zn, suggesting an antagonistic effect between these essential elements. Although we do not know whether this is due to the interaction of Se with I, or simply to the higher-Se accumulation, the Se/Zn interaction has been reported in vegetables following soil enrichment with Zn and Se, since Se alone depressed Zn while Zn

Table 2. Yolk mineral content of commercial eggs according to the studied categories

Category (*n*)	Element (mean ± SD)						
	Ca (mg/g dw)	Mg (μg/g dw)	Fe (μg/g dw)	Zn (μg/g dw)	Se** (μg/g dw)	Pb (μg/g dw)	Cd* (ng/g dw)
Organic (6)	2.07 ± 0.17	189.8 ± 16.5	120.5 ± 12.0	82.4 ± 5.3	1.38 ± 0.14[(a)]	0.28 ± 0.15	7.38 ± 4.75[(a)]
Free range (6)	2.09 ± 0.47	189.7 ± 17.6	119.1 ± 8.2	77.6 ± 6.0	1.04 ± 0.23	0.26 ± 0.10	10.43 ± 2.29
Barn (6)	1.93 ± 0.13	192.7 ± 10.7	130.1 ± 6.9	78.1 ± 5.6	1.04 ± 0.17	0.32 ± 0.07	14.50 ± 3.32
Battery (9)	2.00 ± 0.2	200.8 ± 12.1	128.5 ± 13.2	80.2 ± 4.1	1.04 ± 0.19	0.26 ± 0.15	11.18 ± 4.98
Farmyard (6)	1.88 ± 0.26	213.0 ± 25.8	120.9 ± 17.3	81.4 ± 4.6	0.70 ± 0.15[(b)]	0.16 ± 0.06	8.40 ± 4.75[(b)]

*$F = 2.90$, $p < 0.05$; [(a), (b)] $p < 0.05$ vs. the barn category.
**$F = 9.30$, $p < 0.001$; [(a), (b)] $p < 0.05$ vs. all the other categories.

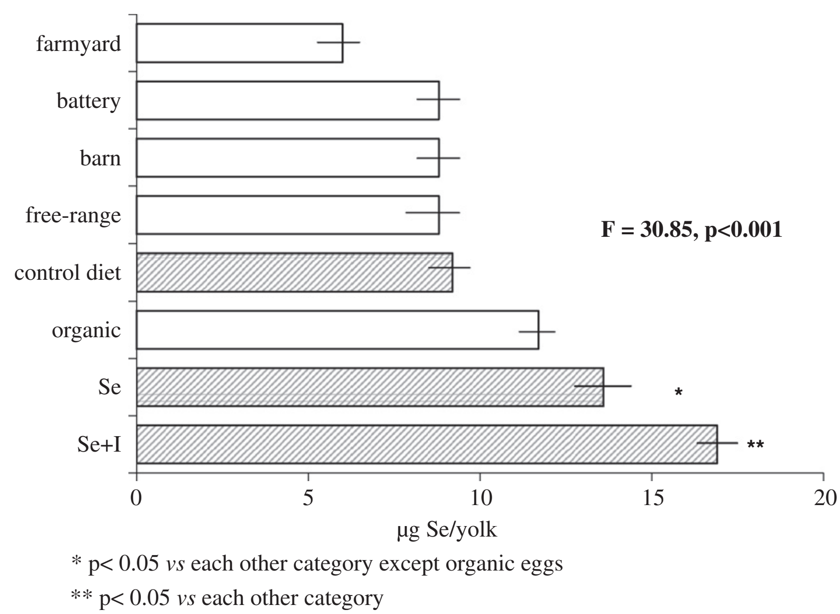

Fig. 2. Se amount per yolk in experimental (grey bars) and commercial eggs (white bars) according to their content (mean ± SD).

alone depressed Se assimilation [37]. An inverse relation between Se and Zn was also reported in an Italian longitudinal study on human milk from full-term and preterm mothers [50]. On the other hand, the similarity of Zn and Se, which are both 4th period elements with similar atomic numbers, may explain their antagonism.

Chickenfeed supplementation with sodium selenite in our experiments caused an accumulation of the Se in egg yolk not in ovalbumen. This finding is in line with evidence that the chemical forms of Se can influence its distribution as selenomethionine and selenized yeast is mainly deposited in egg white, while inorganic Se or non-selenomethionine compounds are mainly deposited in yolk [21–24]. In addition, inorganic forms of Se are added to the basic diet of our hens, as sodium selenite and selenate are the only forms admitted to satisfy Se needs of poultry in Europe [43]. As the basic level of Se in yolk of experimental eggs was similar to that found in commercial eggs, we confirmed that inorganic Se compounds actually are the chemical forms used in poultry feeding. For all these considerations and the limited amount of Se and other elements in ovalbumen, we did not conduct trace element analyses on this matrix. Although it is well-known that Se in yolk, white and total egg varies in the literature [51], the lower Se in ovalbumen was always confirmed. Basic values reported in experimental studies ranged from 0.10 to 0.474 μg/g for yolk and from 0.039 to 0.087 μg/g for fresh mass in ovalbumen [17,20,51–53]. In dry weight, Se in yolk ranged from 0.71 to 1.34 and in white from 0.56 to 0.75 μg/g dw, due to the higher water content of ovalbumen [24,38,51]. This variability was also confirmed when values were reported as Se content for egg, ranging from 5 to 18.04 μg/egg [24,27,28,54].

The reported results were obtained by increasing the basic diet containing 0.4 μg/g of Se, according to the CE regulation establishing a maximum content of 0.5 μg/g, up to 1 μg/g in the treatment groups. The selected concentration is far below the toxic levels of 10.0 μg/g dw for laying hens [55]. No significant negative effects on egg production or characteristics were observed, and no signs of toxicity were observed in treated hens [29]. Under these experimental conditions, Se concentration rose from 1.08 to 1.60–1.98 μg/g and the results are in agreement with other studies using sodium selenite as Se source [24,31,53,56,57].

Eggs from free-range, barn and battery-grown hens, characterized by different systems of production but identical diets, exhibited Se content similar to that found in the non-treated experimental group (1.04 μg/g). Instead, a difference was noted in farmyard and organic egg categories, having different diets. Our farmyard hens were nourished with wheat or corn with no other additions, and as a result the yolks were poorer in Se and toxic elements, particularly Cd. The reason for a lower Se content in yolks may depend on the fact that the major seleno-compound in corn is selenomethionine, known to deposit mainly in egg white [21,58]. Interestingly, Robberecht et al. [51] reported similar differences, with a slightly lower yolk Se content in farm eggs compared with commercial eggs (0.74 vs. 0.90 μg/g dw). The lower content of Pb and Cd may result from a non-industrial chickenfeed processing, as feed manipulation can introduce toxic substances. The lower Cd content in organic eggs could depend on a lower Cd contamination of the soil and the ecosystem on which organic crops and livestock are raised, together with the absence of synthetic pesticides and chemical fertilizers which may contain amounts of toxic metals such as Cd [59–61]. This finding and the higher-Se content, which resembles that found in Se-enriched eggs, is of particular interest as organic commercial products are receiving growing attention in industrialized countries. The reason for the higher-Se content of these eggs could depend on a higher-Se addition in the diet and/or on the geographic area where organic wheat is grown [62].

Our study indicates that eggs experimentally enriched in Se can be a good nutritional product to help achieve the daily recommended amount of this antioxidant. From our results, one commercial egg can provide from 6.0 to 11.7 μg/yolk of Se, whereas an Se-enriched egg can provide 13.6 μg/yolk Se, corresponding to 24.8% of the recommended intake in Europe (55 μg/d) or 19.4% in the USA (70 μg/d). This intake is of relevance especially among certain categories of people at risk of Se deficiency such as the elderly [12,30].

Se-enriched food may be a convenient and easy way to improve Se nutrition in humans, but concomitant enrichment with iodine is always more common. A novel finding in our study is that the association of Se with I increases the yolk Se content and counteracts the assimilation of noxious elements like Cd and Pb, but a possible negative interference with essential elements like zinc was disclosed. Thus, the introduction of Se + I fortified eggs in the market requires attention pending in-depth investigations to clarify the interrelationships between Se, I and Zn.

References

[1] Schwarz K, Foltz CM. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J Am Chem Soc 1957;79:3292–3.

[2] Rotruck JT, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science 1972;179:588–90.

[3] Brown KM, Arthur JR. Selenium, selenoproteins and human health: a review. Public Health Nutr 2001;4:593–9.

[4] Yu MW, Horng IS, Chiang YC, Liaw YF, Chen CJ. Plasma selenium levels and the risk of hepatocellular carcinoma among men with chronic hepatitis virus infection. Am J Epidemiol 1999;150:367–74.

[5] Brooks JD, Metter EJ, Chan DW, Sokoll LJ, Landis P, Nelson WG, et al. Plasma selenium level before diagnosis and the risk of prostate cancer development. J Urol 2001; 166:2034–8.

[6] Beck MA, Levander OA, Handy J. Selenium deficiency and viral infection. J Nutr 2003;133:1463S–7S.

[7] Lyons G, Stangoulis J, Graham R. High-selenium wheat: biofortification for better health. Nutr Res Rev 2003;16: 45–60.

[8] Frost DV. Why the level of selenium in the food chain appears to be decreasing. In: Combs GF, Levander OA, Spallholz JE, editors. Selenium in biology and medicine, part A. New York: AVI Van Nostrand; 1987. p. 534–47.

[9] Arthur JR. Selenium supplementation: does soil supplementation help and why? Proc Nutr Soc 2003;62:393–7.

[10] Lyons GH, Judson GJ, Ortiz-Monasterio I, Genc Y, Stangoulis JC, Graham RD. Selenium in Australia: selenium status and biofortification of wheat for better health. J Trace Elem Med Biol 2005;19:75–82.

[11] Broadley MR, White PJ, Bryson RJ, Meacham MC, Bowen HC, Johnson SE, et al. Biofortification of UK food crops with selenium. Proc Nutr Soc 2006;65:169–81.

[12] Bargellini A, Caselgrandi E, Voglioso I, Borella P. Micro e macroelementi essenziali nella dieta di diversi gruppi residenti in comunità. Tec Sanit 1999;35:22–6.

[13] Milner JA. Functional foods: the US perspective. Am J Clin Nutr 2000;71:1654S–9S.

[14] Roberfroid MB. Concepts and strategy of functional food science: the European perspective. Am J Clin Nutr 2000; 71:1660S–4S.

[15] Combs GF. Selenium in global food systems. Br J Nutr 2001;85:517–47.

[16] Hasler CM. The changing face of functional foods. Am J Coll Nutr 2000;19:499S–506S.

[17] Richards MP. Trace mineral metabolism in the avian embryo. Poult Sci 1997;76:152–64.

[18] Kilic Z, Acar O, Ulasan M, Ilim M. Determination of lead, copper, zinc, magnesium, calcium and iron in fresh eggs by atomic absorption spectrometry. Food Chem 2002;76:107–16.

[19] Skrivan M, Skrivanova V, Marounek M. Effects of dietary zinc, iron and copper in layer feed on distribution

of these elements in eggs, liver, excreta, soil and herbage. Poult Sci 2005;84:1570–5.

[20] Dobrzanski Z, Gorecki H, Chojnacka K, Gorecka H, Synowiec M. Effect of dietary humic preparations on the content of trace elements in hens' eggs. Am J Agric Biol Sci 2007;2:234–40.

[21] Latshaw JD, Osman M. Distribution of selenium in egg white and yolk after feeding natural and synthetic selenium compounds. Poult Sci 1975;54:1244–52.

[22] Swanson CA. Comparative utilization of selenite, selenomethionine, and selenized yeast by the laying hen. Nutr Res 1987;7:529–37.

[23] Cantor AH, Straw ML, Ford MJ, Pescatore AJ, Dunlap LK. Effect of feeding organic selenium in diets of laying hens on egg selenium content. In: Sim JS, Nakai S, Guenter W, editors. Egg nutrition and biotechnology. Wallingford, UK: CAB International; 2000. p. 473–6.

[24] Jiakui L, Xiaolong W. Effect of dietary organic versus inorganic selenium in laying hens on the productivity, selenium distribution in egg and selenium content in blood, liver and kidney. J Trace Elem Med Biol 2004;18: 65–8.

[25] Garwin JL, Morgan JM, Stowell RL, Richardson MP, Walker MC, Capuzzi DM. Modified eggs are compatible with a diet that reduces serum cholesterol concentrations in humans. J Nutr 1992;122:2153–60.

[26] Farrell DJ. Enrichment of hen eggs with n-3 long-chain fatty acids and evaluation of enriched eggs in humans. Am J Clin Nutr 1998;68:538–44.

[27] Surai PF, MacPherson A, Speake BK, Sparks NH. Designer egg evaluation in a controlled trial. Eur J Clin Nutr 2000;54:298–305.

[28] Surai PF, Sparks NHC. Designer eggs: from improvements of egg composition to functional food. Anim Feed Sci Technol 2001;12:7–16.

[29] Rizzi L, Simioli M, Bochicchio D, Parazza P. The effects of omega-3 fatty acids, iodine and selenium supplementation of laying hen feed on the egg quality. In: Baeza E Fernandez X, editor. Quality of eggs and egg products. Proceedings of the XVI European symposium on the quality of eggs and egg products. France: Saint Brieuc-Ploufragan; 2003. p. 290–6.

[30] Bourre JM, Galea F. An important source of omega-3 fatty acids, vitamins D and E, carotenoids, iodine and selenium: a new natural multi-enriched egg. J Nutr Health Aging 2006;10:371–6.

[31] Pan C, Huang K, Zhao Y, Qin S, Chen F, Hu Q. Effect of selenium source and level in hen's diet on tissue selenium deposition and egg selenium concentrations. J Agric Food Chem 2007;55:1027–32.

[32] Lyons GH, Stangoulis JC, Graham RD. Exploiting micronutrient interaction to optimize biofortification programs: the case for inclusion of selenium and iodine in the HarvestPlus program. Nutr Rev 2004;62:247–52.

[33] Klotz LO, Kroncke KD, Buchczyk DP, Sies H. Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. J Nutr 2003;133:1448S–51S.

[34] Nolan TD, Brown D. The influence of elevated dietary zinc, selenium, and their combination on the suppressive effect of dietary and intraperitoneal cadmium on egg production in laying hens. J Toxicol Environ Health 2000; 60:549–65.

[35] Skowerski M, Jasik K, Konecki J. Effects of interaction between cadmium and selenium on heart metabolism in mice: the study of RNA, protein, ANP synthesis activities and ultrastructure in mouse heart. Med Sci Monit 2000; 6:258–65.

[36] Xiao P, Jia XD, Zhong WJ, Jin XP, Nordberg G. Restorative effects of zinc and selenium on cadmium-induced kidney oxidative damage in rats. Biomed Environ Sci 2002;15:67–74.

[37] He PP, Lv XZ, Wang GY. Effects of Se and Zn supplementation on the antagonism against Pb and Cd in vegetables. Environ Int 2004;30:167–72.

[38] Stibilj V, Vadnjal R, Kovac M, Holcman A. The effect of dietary arsenic additions on the distribution of selenium and iodine in eggs and tissues of laying hens. Arch Environ Contam Toxicol 2004;46:275–80.

[39] Kohrle J. Thyroid hormone deiodination in target tissues – a regulatory role for the trace element selenium? Exp Clin Endocrinol 1994;102:63–89.

[40] Hotz CS, Fitzpatrick DW, Trick KD, L'Abbe MR. Dietary iodine and selenium interact to affect thyroid hormone metabolism of rats. J Nutr 1997;127: 1214–8.

[41] Cocchi M, Venturi S. Iodide, antioxidant function and omega-6 and omega-3 fatty acids: a new hypothesis of a biochemical cooperation? Prog Nutr 2000;2:15–9.

[42] Thomson CD, McLachlan SK, Grant AM, Paterson E, Lillico AJ. The effect of selenium on thyroid status in a population with marginal selenium and iodine status. Br J Nutr 2005;94:962–8.

[43] Council Directive 70/524/EEC. List of the authorized additives in feedingstuffs 2004/C 50/01. Off J Eur Union C50/1.

[44] Olson OE, Palmer IS, Cary EE. Modification of the official fluorometric method for selenium in plants. J Assoc Off Anal Chem 1975;58:117–21.

[45] Borella P, Bargellini A, Caselgrandi E, Menditto A, Patriarca M, Taylor A, et al. Selenium determination in biological matrices. Microchem J 1998;58:325–36.

[46] Flynn A, Moreiras O, Stehle P, Fletcher RJ, Muller DJ, Rolland V. Vitamins and minerals: a model for safe addition to foods. Eur J Nutr 2003;42:118–30.

[47] Diplock AT, Watkins WJ, Hewison M. Selenium and heavy metals. Ann Clin Res 1986;18:55–60.

[48] Whanger PD. Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. J Trace Elem Electrolytes Health Dis 1992;6:209–21.

[49] Jamba L, Nehru B, Bansal MP. Selenium supplementation during cadmium exposure: changes in antioxidant enzymes and the ultrastructure of the kidney. J Trace Elem Exp Med 1997;10:233–42.

[50] Perrone L, Di Palma L, Di Toro R, Gialanella G, Moro R. Interaction of trace elements in a longitudinal study of human milk from full-term and preterm mothers. Biol Trace Elem Res 1994;41:321–30.

[51] Robberecht H, Benemariya H, van Dael P, Deelstra H. Mineralization procedures and determination of selenium

in egg white, egg yolk and shells of different eggs. Belg J Food Chem Biotech 1987;42:147–53.
[52] Surai PF. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. Br Poult Sci 2000;41:235–43.
[53] Paton ND, Cantor AH, Pescatore AJ, Ford MJ, Smith CA. The effect of dietary selenium source and level on the uptake of selenium by developing chick embryos. Poult Sci 2002;81:1548–54.
[54] Yannakopoulos A, Tserveni-Gousi A, Christaki E. Enhanced egg production in practice: the case of Bio-Omega-3 egg. Int J Poult Sci 2005;4:531–5.
[55] Piva G, Pietri A, Prandini A, Marzoli A, Morlacchini M. Cobalto, Selenio, Iodio, Manganese: considerazioni sull'impiego di questi oligoelementi in alimentazione animale. Assalzoo 1993;55. 〈http://www.assalzoo.it/〉.
[56] Utterback PL, Parsons CM, Yoon I. Effect of supplementing selenium yeast in diets of laying hens on egg selenium content. Poult Sci 2005;84:1900–10.
[57] Payne RL, Lavergne TK, Southern LL. Effect of inorganic versus organic selenium on hen production and egg selenium concentration. Poult Sci 2005;84:232–7.
[58] Sheng Z, Jiakui L, Haijian L, Bin L, Xiaolong W. Investigation of selenium content of eggs sampled from three major commercial cities along the lower reaches of the Changjiang River. Res Vet Sci 2002;72:7–9.
[59] Srikant R, Vasant Rao, Venugopal NBRK. The effects of application of phosphatic fertilizer on cadmium concentration in ground waters around vineyards of Hyderabad. Environ Monit Assess 1994;33:71–4.
[60] Huang B, Kuo S, Bembenek R. Cadmium uptake by lettuce from soil amended with phosphorus and trace element fertilizers. Water Air Soil Pollut 2004;147:109–27.
[61] Lugon-Moulin N, Ryan L, Donini P, Rossi L. Cadmium content of phosphate fertilizers used for tobacco production. Agron Sust Dev 2006;26:151–5.
[62] Finley JW. Bioavailability of selenium from foods. Nutr Rev 2006;64:146–51.