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Developing an egg model for selenium nanoparticle testing

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ABSTRACT

Selenium nanoparticles (SeNPs) are gaining attention due to their enhanced bioavailability and potential use in functional food development. This preliminary study examined the ability of red and grey SeNPs to penetrate the eggshell and enrich internal egg components with selenium through external immersion treatments. Commercial eggs were treated with two concentrations (50 and 100 mg L^{-1}) of red and grey SeNPs and analysed for selenium content in raw and boiled albumen and yolk. Results showed increased selenium deposition in SeNP-treated eggs compared to untreated controls, with the highest accumulation observed in the yolk. Red SeNPs, owing to their smaller size and amorphous structure, exhibited greater bioavailability and deposition efficiency than grey SeNPs. Boiling led to variable changes in selenium content, suggesting possible heat-induced structural transformations or retention effects. Due to the limited

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sample size and uncontrolled egg source, these findings should be interpreted as exploratory. Nonetheless, this study provides initial evidence that SeNPs can cross physical egg barriers and may inform future strategies for egg biofortification and future applications in animal nutrition and food science.

KEYWORDS

selenium nanoparticles, antioxidant, density, Se content, enrichment, nanoscale, egg, porosity, shell, yolk, albumen

1. INTRODUCTION

Selenium is an essential trace mineral, plays a fundamental role in various biological processes, and exhibits antioxidant properties. Selenium nanoparticle (SeNP), which raises to selenium at the nanoscale, has distinct characteristics that attracted major interest in the scientific community to study and apply in various areas such as health, agriculture, and environmental remediation (Zhang et al., 2001; Cai et al., 2012; El Deep et al., 2016). These unique properties are due to their nanoscale size and high reactive surface area, leading to improved bioavailability and enhanced biological activity compared to inorganic forms of selenium (Zambonino et al., 2023).

Nano selenium has been investigated as an eventual nutrient supplement for animal feed and plant fertilisers in the fields of agriculture. It may promote livestock and crop growth, development, and good health (Cai et al., 2012). The potential significance of nano selenium in improving the nutritional quality of food products has also been examined (Nabi et al., 2020). Nano selenium has also been studied for its environmental applications, it has been used to clean up heavy metals such Cu, Zn, Hg, and Cd and pollutant-contaminated water and soil (Kumar and Prasad., 2021). Nano selenium has demonstrated the ability to lower these pollutants' toxicity and environmental impact (El-Ramady et al., 2014). Meng et al. (2019) evaluated the influence of nano selenium supplementation on laying hens in egg production and quality. The results demonstrated an effective relation, as nano selenium treatment improved egg production and several egg quality indicators and antioxidant status in laying hens. This implies that adding nano selenium to laying hen diets has the advantage of enhancing the nutritional quality of egg. It specifically causes a rise in egg selenium content and improves critical egg quality parameters such as the Haugh unit, albumen pH, yolk index, yolk colour, and egg weight loss. Additionally, these studies illustrate that nano selenium supplements can improve eggs' antioxidant profile, ovalbumin, ovotransferrin, phosvitin, phospholipids, vitamin A, vitamin E, carotenoid, and selenium contents, and increase their overall nutritional value (Omri et al., 2019).

The avian egg, serving as a reproductive tool and a vital human food source, varies in size and form between bird species. The dimensions and internal quality of eggs hold significance for both culinary and hatching purposes (Şekeroğlu and Altuntaş, 2009), encompassing three essential components: yolk, albumen, and shell. The eggshell performs an essential function in maintaining a delicate equilibrium throughout embryo development. It must be thick and robust enough to shield the embryo from bodily harm, while remaining thin and flexible enough not to interfere during hatch. The shell should have an appropriate pore percentage to allow oxygen exchange and prevent harmful micro-flora invasion for a good embryonic development

(Narushin and Romanov., 2002). Similarly, while being a rich source of protein, fats, and other nutrients, eggs are an ideal model for studying the permeability of egg membranes to nanoparticles, and the migration behaviour of selenium nanoparticles. This project aims to compare the performance of grey (hexagonal) elemental nano selenium the red (amorphous) nano selenium using egg as a developed model system. The key parameters measured include mass, selenium content, and their impact on both raw and boiled eggs.

2. MATERIALS AND METHODS

The study was conducted at the University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Animal Science, Biotechnology and Nature Conservation, Department of Animal Husbandry, Nanofood laboratory, Hungary.

2.1. Reagents and standards

Sodium selenite, vitamin C, nitric acid 65% (AR grade), hydrogen peroxide, and hydrochloric acid 37% (AR grade) were obtained from VWR, International Ltd. (Lutterworth, Leics, UK). Sodium borohydride 98% (AR grade) was purchased from Acros Organics (Geel, Belgium).

2.2. Selenium production

Red and grey nanoparticles of selenium were prepared according to the method described by Khandsuren and Prokisch (2021). Chemical synthesis of nano selenium involves the reduction of sodium selenite to elemental selenium nanoparticles by reducing agents, like ascorbic acid. 0.2 g of sodium selenite (Na_2SeO_3) was dissolved in 100 mL water to prepare stock solution at 1,000 mg L^{-1}, and 1% of ascorbic acid solution was added to sodium selenite solution (v/v). It was kept for 30 min at room temperature for sodium selenite reduction and the formation of nanoselenium particles. After the reaction, the solution obtained is red SeNPs, and heating that solution at 85 °C for 30 min produces grey nano selenium as shown in Fig. 1.

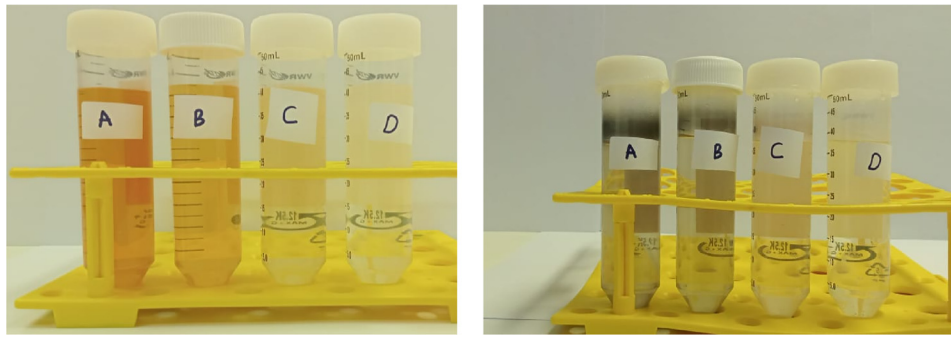

Fig. 1. Nanoparticle selenium solutions; left: Red selenium and right: Grey selenium; A = 100 mg L^{-1}, B = 50 mg L^{-1}, C = 10 mg L^{-1}, D = 1 mg L^{-1}

2.3. Experiment design

A total of 30 commercially sourced chicken eggs (S-Budget brand; Class A, medium size, 53–63 g per egg) were used in the trial. The eggs were randomly divided into three treatments: control (0 mg L^{-1}), 50 mg L^{-1}, and 100 mg L^{-1} of red or grey nano-selenium. The treatment solutions were applied externally by immersing each egg separately in 100 mL of the respective solution, and keeping for 8 days in the fridge at 4 °C. By the end of the experiment, the eggs were divided into 2 groups; the first was measured immediately, and the other group was examined after boiling the eggs for 2 h at 80 °C in the oven in their treatment solutions to examine thermal effects on selenium distribution. The eggs were crushed and separated into yolk, white, and eggshell. The experimental design is summarised in Fig. 2.

2.4. Sample preparation

The eggs were weighed before and after the experiments presented as mean ± SD.

The method described by Muhammad et al. (2021) was employed with modifications to digest the samples by using 5 mL of nitric acid HNO_3 (96%) and 3 mL of H_2O_2 (30%) with 1 g of each egg fraction for 10 min in the oven at 50 °C. Later these solutions were diluted with 50 mL of distilled water, filtered through 0.45 µm filter, and diluted 100 times with 3M HCl. These prepared samples were subsequently analysed for selenium content by Atomic Fluorescence Spectrophotometer (AFS) Millennium Excalibur 10.055 (PSA, Orpington, UK).

2.5. Statistical analyses

All statistical tests were conducted using the MINITAB 2019 and the results are reported as mean values ± standard error of the mean. The differences between the Se treated groups were analysed by a one-way analysis of variance (ANOVA), followed by a Fisher pairwise comparisons test when a statistically significant ($P < 0.05$) result was observed among the different treatment groups.

3. RESULTS AND DISCUSSION

Table 1 presents egg mass data before and after nano-selenium exposure. In both raw and boiled eggs, treatment with red and grey SeNPs at 50 and 100 mg L^{-1} generally maintained or

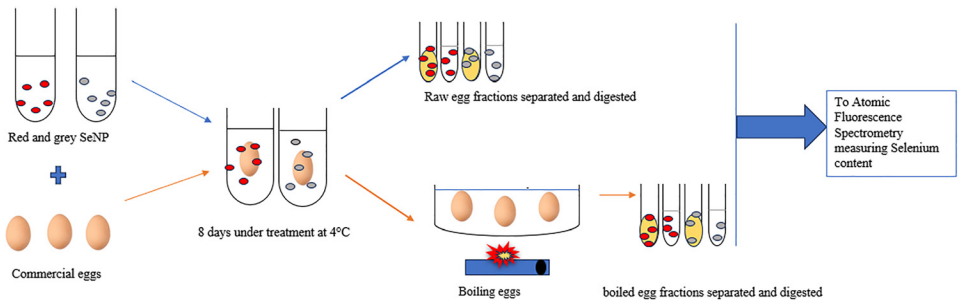

Fig. 2. Diagram of the egg model experiment to test selenium nanoparticles

Table 1. Means of egg mass before and after treatment with two different forms of nano selenium and doses for 8 days ($n = 30$)

Nano selenium	Egg status	Dose (mg L^{-1})	Initial egg mass (g)	Final egg mass (g)
Red SeNPs	Raw	0	48 ± 5	47 ± 5
		50	50 ± 2	54 ± 5
		100	52 ± 1	53 ± 1
Grey SeNPs		50	49 ± 2	51 ± 1
		100	50 ± 2	50 ± 2
Red SeNPs	Boiled	0	42 ± 2	43 ± 2
		50	44 ± 0.9	44 ± 0.9
		100	45 ± 2	47 ± 5
Grey SeNPs		50	48 ± 2	50 ± 2
		100	45 ± 1	46 ± 1

insignificantly increased mass compared to the control. This suggests that SeNP immersion did not adversely affect egg water content or structure.

Selenium levels (Table 2) significantly increased in most nano-selenium-treated groups, particularly those exposed to red SeNPs. Eggs were immersed externally in 100 mL of either 50 or 100 mg L^{-1} SeNP solutions for 8 days, resulting in marked selenium deposition in both yolk and albumen, raw and boiled, compared to controls. The highest value was recorded in the raw albumen of the red SeNP 50 mg L^{-1} group (RW50), reaching 5459.4 μg kg^{-1}, while the control albumen showed the lowest (52.2 μg kg^{-1}). Notably, selenium levels exhibited high variability, especially in RW50 and RW100 groups, as reflected by large standard errors. This may be due to inconsistent nanoparticle adhesion, differences in shell permeability, or SeNP aggregation, all of which are affected by solution pH, temperature, and stability.

Interestingly, visible changes further highlighted the different behaviour of SeNPs, as after 8 days of treatment, a discernible layer formed on the eggshell surface due to continuous nanoparticle precipitation. Red SeNP-treated eggs developed a thin reddish surface layer, as depicted in Fig. 3b and d, while the grey SeNPs formed a dark and thick layer, illustrated in Fig. 3a and c. After boiling, the grey treatment group displayed a noticeable colour change, but the red ones maintained their original white albumen colour as presented in Fig. 3e and f.

Our results demonstrate that eggs treated with selenium nanoparticles, both red and grey forms, showed significantly higher selenium content compared to the control group, confirming the ability of SeNPs to penetrate the eggshell and deposit selenium into internal egg components. This is consistent with the nanoscale size of SeNPs, which likely facilitates their passage through the eggshell pores (~0.2–0.25 μm) and biological membranes (Wang et al., 2016; Zhang et al., 2023).

Among the two SeNP types, red selenium nanoparticles exhibited higher selenium deposition in both raw and boiled eggs than grey SeNPs. This difference can be attributed to the distinct physicochemical properties of the SeNPs: red SeNPs are typically amorphous and smaller (50–100 nm), which enhances their bioavailability and cellular uptake (Khandsuren and Prokisch., 2021). In contrast, grey SeNPs possess a crystalline hexagonal structure that may promote aggregation, limiting their penetration and transport into egg compartments.

Table 2. Selenium levels in raw and boiled egg yolk and white for the red and grey nano selenium treatments and the control group

Treatment	CW	CY		RW50	RW100	RY50	RY100	GW50	GW100	GY50	GY100
Se content ($\mu g\ kg^{-1}$)	52 ± 4^{c}	296 ± 38^{b}	Raw	$5{,}460 \pm 4{,}880^{a}$	$3{,}210 \pm 1{,}970^{a,b}$	498 ± 197^{b}	516 ± 191^{b}	74 ± 16^{c}	53 ± 8^{c}	303 ± 18^{b}	302 ± 15^{b}
			Boiled	392 ± 63^{b}	578 ± 290^{b}	779 ± 40^{b}	$3{,}865 \pm 2{,}943^{a,b}$	526 ± 187^{b}	311 ± 129^{b}	695 ± 43^{b}	663 ± 36^{b}

C: control; G: grey SeNPs; R: red SeNPs; Y: yolk; W: white. Means with different letters are significantly different.

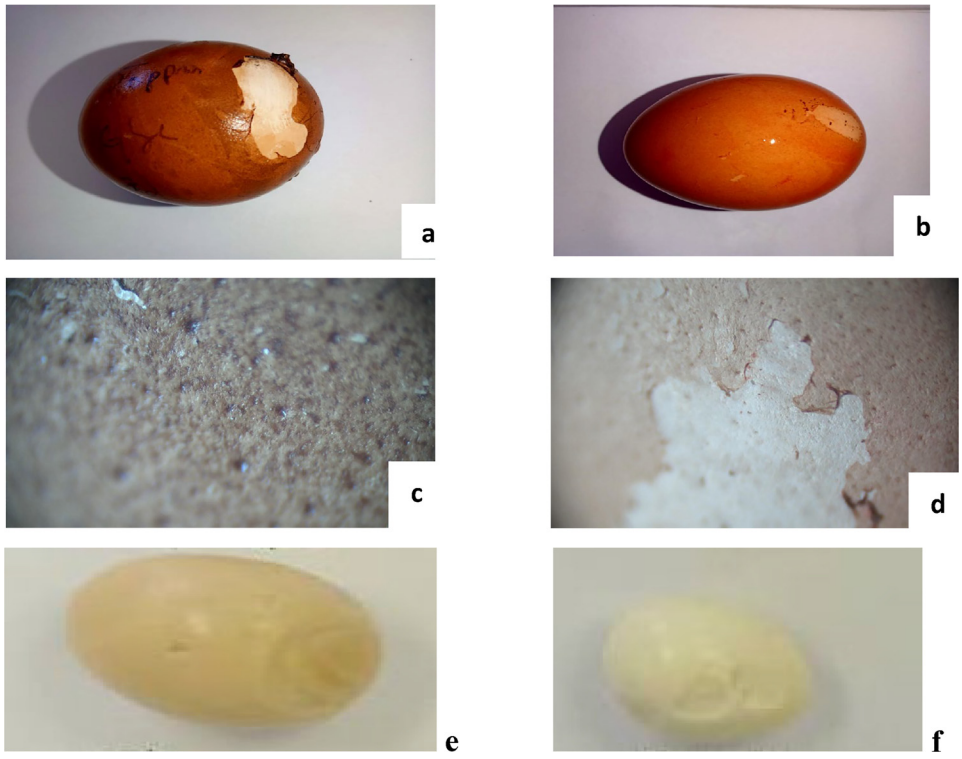

Fig. 3. Eggshell under treatment with SeNPs solutions at 50 mg L^{-1}. (a, c): Grey nano selenium; (b, d): Red nano selenium layer; (c, d): Eggshell photo under microscope; Cracked boiled eggs at 50 mg L^{-1} with grey (e) and red (f) nano selenium solutions

Boiling affected selenium content variably across treatments. The red selenium levels in the albumen decreased after boiling, possibly due to thermal-induced volatilisation, oxidation, or structural changes in the SeNPs, such as aggregation, which reduce selenium bioavailability (Dong et al., 2021; Farooq et al., 2024). However, grey SeNPs showed increased selenium in yolk after boiling compared with with raw samples of the same doses, which might be explained by heat-induced expansion of eggshell pores facilitating deeper SeNP penetration into the yolk, or protein coagulation trapping selenium compounds within egg compartments (Cree and Pliya., 2019; Oliveira et al., 2020).

The non-linear dose response observed – where lower SeNP concentrations (50 mg L^{-1}) sometimes resulted in higher selenium accumulation than higher doses (100 mg L^{-1}) – may reflect biological saturation mechanisms or nanoparticle aggregation at higher concentrations, which reduces their bioavailability (Dong et al., 2021). Additionally, individual variation among samples could contribute to the observed variability, as suggested by the wide standard deviations.

Overall, these findings support that SeNPs can enhance selenium deposition in eggs, with red SeNPs showing superior bioavailability compared to grey SeNPs. The results also indicate that SeNPs are capable of penetrating the internal barriers – such as the eggshell. The impact of boiling on selenium retention warrants further study, especially regarding the thermal stability of different SeNP species and their interaction with egg components.

4. CONCLUSIONS

This study suggests that selenium nanoparticles (SeNPs), particularly in their red amorphous form, may enhance selenium deposition in eggs when applied externally. The findings indicate that nanoparticle properties – such as size, structure, and chemical form – could influence their ability to penetrate the eggshell and interact with internal egg components. Red SeNPs appeared to result in higher selenium accumulation than the grey SeNPs, potentially due to their smaller particle size and enhanced mobility.

Boiling introduced further complexity, potentially altering selenium content through heat-induced aggregation, volatilisation, or entrapment within protein matrices. While these preliminary observations provide useful insights into SeNP behavior in egg systems, the small sample size and uncontrolled egg source variability limit the strength of the conclusions. Therefore, these results should be viewed as hypothesis-generating and call for further research under controlled conditions with larger sample sizes to validate the trends observed and better understand the mechanisms involved in selenium nanoparticle uptake and retention in eggs.

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