

Opinion

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Abstract

In recent years, nanoparticles have become a fashionable subject of research due to their sizes, shapes, and unique intrinsic physicochemical properties. In particular for the last 5 years, nano-Se has received tremendous attention in terms of its production, characteristic, and possible application for poultry/animal science and medical sciences. Indeed, Nano-Se is shown to be a potential source of Se for poultry/animal nutrition. However, there is an urgent need to address the questions related to nano-Se absorption, assimilation, and metabolism. It is not clear at present if major biological effects of nano-Se are due to Se-protein synthesis, direct antioxidant/prooxidant effects, or both. It is necessary to understand how metallic nano-Se can be converted into H₂Se and further to SeCys to be incorporated into selenoproteins. The aforementioned issues must be resolved before nano-Se finds its way to animal/poultry production as a feed supplement and clearly this subject warrants further investigation.

Marcovic *et al.* (2018) recently published an excellent review in *Animal Health Research Reviews* (Volume 19, pp. 166–176), which describes important roles of Se in poultry nutrition. They presented a review of data related to nano-Se and used them as an example of successful nanotechnology application in poultry production. In particular, they suggested that “Nano-Se and organic Se possess at least comparable (and sometimes improved) efficiency to inorganic Se in upregulating selenoenzymes, and have higher bioavailability and lower toxicity”.

In recent years, nanoparticles have become a center of research attraction due to their sizes, shapes, and unique intrinsic physicochemical properties (Bai *et al.*, 2018; Ramachandiraiah *et al.*, 2018). In particular for the last 5 years, nano-Se has received tremendous attention in terms of its production, characteristic, and possible application for poultry/animal science and medical sciences (Pelyhe and Mézes, 2013; Griffin *et al.*, 2017; Skalickova *et al.*, 2017; Guan *et al.*, 2018; Hosnedlova *et al.*, 2018; Khurana *et al.*, 2019; Patra and Lalhriatpuii, 2020). However, there are some unresolved issues and unanswered questions which should be addressed by scientific community before nano-Se can find its way as a nutritional supplement or a drug in agriculture and medicine/veterinary medicine. We would like to summarize them as follows. First, it is necessary to mention several fundamental issues which are important for the understanding of molecular mechanisms of nano-Se action.

Fundamentals of Se action in poultry (Surai and Fisinin, 2014; Surai, 2018; Surai et al., 2018; Surai and Kochish, 2019)

- Biological activity of Se is related to synthesis of 25 selenoproteins and their actions;
- Selenoprotein synthesis depends on the level of stress (internal and/or external) and Se supply. Some selenoproteins performing housekeeping functions have constant low expression and are not stress-inducible; however, most Se-proteins are stress- and Se-inducible;
- Se-proteins are not synthesized in advance and their expression and synthesis reflects the current situation in the cell/tissue. Only optimal Se supply could induce optimal selenoprotein synthesis response;
- In most cases, induction of Se-proteins by nutritional Se supplements takes place only if Se level in the diet is not adequate/low;
- Se excess, beyond current requirement, would not upregulate Se-protein synthesis and could have an opposite toxic effect;
- Poultry Se requirement under physiologic conditions is quite low and it is set at 0.15–0.2 mg kg⁻¹. It is known that dietary feed ingredients in the final diet could provide Se at 0.1–0.3 mg kg⁻¹, while commercial diets are usually supplemented with 0.3 mg Se kg⁻¹, independent of the background Se level, to provide a safety margin under stress conditions when Se requirement can be dramatically increased, but feed consumption usually decreased;

- Organic selenium supplements, containing SeMet as an active compound, have been shown to be more effective in comparison with sodium selenite/selenate and are important parts of the ‘insurance policy’ strategy to build Se reserves in the body (muscles), which can be used in stress conditions to maintain optimal Se-protein expression;
- Among organic Se sources in poultry nutrition, Se-yeast, SeMet, and OH-SeMet found their way into commercial poultry feed. In particular, a new Se source, the hydroxy-analog of selenomethionine (OH-SeMet), combined advantages of both Se-yeast (as a stable Se source) and SeMet (as highly concentrated Se source (Surai *et al.*, 2018));
- Se toxicity in modern commercial poultry production is not a problem, because detrimental effects of Se for poultry starts at a dosage exceeding legal limits of supplementation by more than 3-fold;
- Legal limit of Se supplementation in poultry diets is 0.3 ppm in the USA, and in Europe the total Se of the feed should not exceed 0.5 ppm. It should be mentioned that supplementing organic selenium can result in higher Se levels in edible tissues and milk. Consequently, in the European Union the authorized maximum value for supplementing organic selenium as feed additive is lower (0.2 mg kg^{-1}) than for inorganic compounds (0.3 mg kg^{-1} ; Surai *et al.*, 2018);
- All dietary forms of Se are converted to the common intermediate, namely H_2Se , with following SeCys synthesis and its incorporation into selenoproteins.

The main advantages of nano-Se for poultry/animal nutrition include:

- (a) Improved absorption and assimilation/availability;
- (b) Decreased toxicity. However, nano-Se toxicity for various farm animals and poultry, depending on age and conditions of growth, needs more research.

Unresolved questions

- (a) Are major biological effects of nano-Se due to Se-protein synthesis, due to direct antioxidant/prooxidant effects or both?
- (b) How metallic nano-Se can be converted into H_2Se and further to SeCys to be incorporated into selenoproteins?
- (c) Are there long-term negative consequences of nano-Se usage in poultry nutrition?

Discussion

There is a range of publications showing upregulation of Se-protein expression/activity due to nano-Se supplementation (Wang, 2009; Zhou and Wang, 2011; Cai *et al.*, 2012). In general, dose-dependent activation of glutathione peroxidase 1 (GPx1), GPx4, and thioredoxin reductase (TR) by nano-Se was clearly demonstrated (Zhang *et al.*, 2001), similar to other forms of supplemental Se (Surai, 2018). Indeed, nano-Se is shown to improve the antioxidant status of the cell by preventing a reduction of the levels of glutathione (GSH) and superoxide dismutase (SOD) (Hassanin *et al.*, 2013) and GPx activities (Zhou *et al.*, 2009) and preventing increase of malondialdehyde (MDA) concentrations (Hassanin *et al.*, 2013). However, there are no data available in those publications to distinguish between upregulation of Se-protein synthesis due to oxidative stress imposed by nano-Se or due to increased Se provision. It is well known that many

selenoproteins are oxidative stress-regulated. For example, GPx1, GPx4, and TR1 were demonstrated to be upregulated in response to oxidative stress (Touat-Hamici *et al.*, 2014). Furthermore, such a response was more pronounced when Se supply was limiting. The oxidative stress imposed by nano-Se could be easily demonstrated. For example, in various stress models nano-Se was shown to elevate the levels of heat shock protein (HSP-70; Kumar *et al.*, 2014) and HO-1 (Song *et al.*, 2017), vitagenes and main protective molecules under stress conditions (Surai and Fisinin, 2016; Surai and Kochish, 2017; Surai, 2020). Increased SOD activity in chicken tissues (Mohapatra *et al.*, 2014) could also be an adaptive response to stress caused by nano-Se. Recent data on upregulation of transcription factors (e.g. Nrf2) by nano-Se could indicate improvement of antioxidant defences *via* indirect, mild prooxidant effect of nano-Se (Song *et al.*, 2017). The authors showed that the knockdown of Nrf2 significantly blocked the antioxidative effect of nano-Se particles. Therefore, nano-Se (in the form of so-called biogenic nano-selenium particles) was clearly shown to activate the Nrf2–ARE pathway through p38, ERK1/2, and AKT mediated-phosphorylation of Nrf2 to improve the antioxidant function of intestinal epithelial cells (Xiao *et al.*, 2019). Furthermore, in model systems based on prevention of oxidative stress and toxicity by various antioxidants nano-Se behaves as a typical Nrf-2 inducer. For example, co-administration of nano-Se high dose along with di-*n*-butyl phthalate significantly decreased the level of MDA, and also improved GSH concentration and GPx and SOD activities initially compromised by the toxicant treatment (Rashad *et al.*, 2018). Similarly, in male rats treated with lead acetate, oxidative stress was observed in thyroid tissues and nano-Se supplementation restored antioxidant defence mechanisms (GPx, catalase (CAT), SOD, and GSH) and expression of iodothyronine deiodinase type 1 which were compromised due to the lead-acetate treatment (Atteia *et al.*, 2018). Protective effects of nano-Se against oxidative stress and testicular damage induced by free-radical producing chemical bisphenol A (BPA) was also shown (Abdel-Halim *et al.*, 2016). Indeed, as indicated by CAT activity, GSH level, DNA fragmentation, and lipid peroxidation, nano-Se is shown to have a clear protective effects against BPA-induced oxidative stress in rats (Khalaf *et al.*, 2019). It could well be that the protective effect of nano-Se was mediated *via* Nrf2. Furthermore, biogenic Se nanoparticles synthesized by *Lactobacillus casei* were shown to protect the intestinal epithelial barrier function against H_2O_2 -induced oxidative damage via the Nrf2 signaling pathway (Xu *et al.*, 2019). The authors showed that Nrf2 inhibitor (ML385) abolished the protective effect of Se nanoparticles on intracellular reactive oxygen species (ROS) production. Similar protective action is evident for non-traditional antioxidants inducing Nrf2 including carnitine (Surai, 2015a, 2015b, 2015c), taurine (Seidel *et al.*, 2018; Surai *et al.*, 2020), and silymarin (Surai, 2015d). Interestingly, sodium selenite can also trigger Nrf2-mediated protection in Cd-treated chicken hepatocytes with reduction in Cd-induced autophagy and apoptosis as a consequence (Zhang *et al.*, 2017). It is not known at present if the mechanisms of Nrf2 activation by selenite and nano-Se are the same and if they are connected to selenoprotein expression and synthesis. Furthermore, immunomodulating effects of nano-Se could be related to downregulation of another transcription factor, namely NF- κ B, by nano-Se (Miroliac *et al.*, 2011). Furthermore, this effect of nano-Se was similar to that observed when murine macrophage cultures were treated with sodium selenite *in vitro* (Kim *et al.*, 2004). Again, it is not clear if this effect of Se is related to selenoprotein synthesis. However,

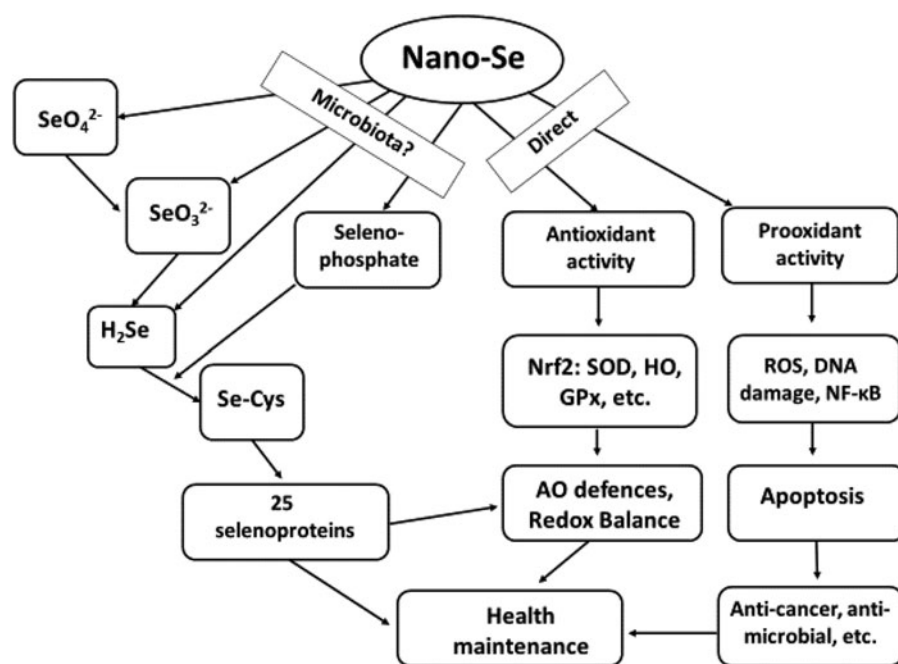


Fig. 1. Possible pathways of nano-Se participation in health maintenance.

when in Se-supplemented rats all significantly changed putative metabolites were studied, it was shown that Se nanoparticles and sodium selenite induced similar dose-dependent changes of the metabolite pattern (Hadrup *et al.*, 2016). In a number of model systems sodium selenite and nano-Se are shown to have similar efficacy.

In addition, anticancer effects and other medical application of nano-Se are related to imposed oxidative stress by inducing ROS production, disruption of mitochondrial membrane potential, and activation of the mitochondria-mediated apoptotic pathway (Khurana *et al.*, 2019). This effect of nano-Se on cancer cells (e.g. H22 hepatic cancer cells, Wang *et al.*, 2014) is similar to that of sodium selenite (Surai, 2006). In fact, both forms of Se cause oxidative stress *in vitro* and *in vivo*. Interestingly, the antibacterial action of SeNPs is also independent of selenoprotein synthesis and could be associated with the production of ROS (Sakr *et al.*, 2018). Indeed, nano-Se was shown to have antimicrobial activities against *Trichophyton rubrum* (Yip *et al.*, 2014), *Candida albicans* (Kheradmand *et al.*, 2014), *Pseudomonas aeruginosa*, *Proteus mirabilis* (Shakibaie *et al.*, 2015), *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella Typhimurium*, *S. Enteritidis* (Khiralla and El-Deeb, 2015), *S. aureus* (Nguyen *et al.*, 2017), and *Toxoplasma gondii* (Keyhani *et al.*, 2020). Interestingly, nano-Se is more effective than sodium selenite with regard to its anti-coccidial, anti-oxidant, and anti-inflammatory role against coccidiosis induced in the jejunum of mice (Alkhudhayri *et al.*, 2018). Nano-Se was also shown to restrict the growth of nanobacteria (Sardarabadi *et al.*, 2019). Therefore, non-selenoprotein action of nano-Se is proven but further research is needed to address the question if both (*via* selenoproteins or direct action) mechanisms are responsible for biological effects of nano-Se.

The question about conversion of nano-Se into selenite/selenate/H₂Se has recently been addressed in our previous publication (Surai *et al.*, 2017) and could be summarized as follows:

- Microbiota in the gut could be responsible for conversion of metallic inert Se⁰ into SeO₃²⁻, SeO₄²⁻, or directly to H₂Se with

following synthesis of SeCys and its incorporation into newly synthesized selenoproteins (Loeschner *et al.*, 2014; Bao *et al.*, 2015). It seems likely that similarities of sulfur and selenium chemistry (Surai, 2006) could help elucidate microbiota-related pathways involved in Se⁰ assimilation.

- A great variety of microbes residing in the chicken/animal gut provides necessary conditions for various Se conversions. For example, when nano-Se particles were incubated with lactic acid bacteria there was a production of organic Se compounds (mainly SeCys and SeMet). At the same time nano-Se particles were partially dissolved and non-metabolically transformed into inorganic selenium (Palomo-Siguero and Madrid, 2017).
- There is a need for further research to prove if the aforementioned nano-Se conversions take place in the gut. In the complex gut ecosystem, a range of microbes could be able to facilitate oxidation/reduction of nano-Se particles, however, this assumption awaits further investigation. In fact, transformation of Se in microorganisms is not yet fully understood. The effect of various forms of Se, including nano-Se, on gut microbiota composition and dynamic also awaits investigation.
- Se can affect microbiota composition and metabolism. In fact, differences in protein expression in *Lactobacillus reuteri* CRL 1101 were observed in the presence of either selenite or SeNPs respect to the control medium. In particular, SeNPs were shown to up-regulate proteins related to nucleotide, vitamins, cofactors, and lipid metabolism, as well as in the transcriptional regulator process (Gomez-Gomez *et al.*, 2019). It was demonstrated that OH-SeMet affected rumen bacteria differently than sodium selenite, resulting in promotion of rumen fermentation (e.g. increasing total volatile fatty acids and the molar proportions of propionate and butyrate but decreasing rumen pH, ammonia content, and the ratio of acetate to propionate), apparent nutrient digestibility and selenium absorption (Wei *et al.*, 2019).

It seems likely that increased availability in comparison with sodium selenite could be a great advantage in some medical applications of Se where direct (non-selenoprotein) action is key for its

efficacy (Menon *et al.*, 2018). However, the same features of nano-Se metabolism and assimilation could be disadvantageous in animal nutrition where the main mechanism of biological activity of Se is mediated *via* selenoprotein synthesis (Surai, 2018). First of all, uncontrolled and enhanced nano-Se penetration via cellular barriers could have negative consequences for animal health. Indeed, when considering nano-Se absorption and its effects on gut health, it is necessary to take into account the so-called Trojan-horse effect, when nanoparticles may have permeation-enhancing properties for other substances in the gut (Fröhlich and Roblegg, 2012). This could be damaging for the gut and organism in general, since there is a range of 'unwanted' compounds/chemicals in the feed and the gut is protective against their absorption. Therefore, nano-Se behavior in the gut warrants further investigation. Second, it is not known in which form Se is accumulated in tissues due to nano-Se supplementation and this raises a question about the long-term consequences of such supplementation. Third, it seems likely that nano-Se cannot build Se reserves in the body in the form of SeMet and therefore the whole concept of organic Se advantages in animal/poultry nutrition (Surai *et al.*, 2018) cannot be applied to nano-Se. Fourth, as it was mentioned above, Se toxicity, in general, is not a problem in modern poultry/animal nutrition. Fifth, nano size of Se particles in the supplement could be a great health hazard for feed mill personnel (Surai, 2018). In addition, based on mode of action nano-Se should be considered as a drug, not a feed/food additive. Finally, Se supplementation in any form to the balanced diet of animals/poultry housed under optimal conditions usually did not affect productive and reproductive performance (Surai, 2006). Furthermore, the promising results and the positive responses of nano-Se in poultry/animal nutrition should not overshadow the possible detrimental consequences of its usage. In particular, nanoparticle behavior under various conditions could differ substantially, and before we understand how to control that behavior, nano-Se usage on a wide industrial scale is in question. In fact, supplementation of nano-Se was shown to have variable responses compared with the other Se sources on production performance of chickens and layers (Patra and Lalhriatpuii, 2020). Suggested pathways of nano-Se participation in animal health maintenance are shown in Fig. 1.

Conclusion

For the last 5 years nano-Se has received tremendous attention in terms of its production, characteristic, and possible application for medical sciences. Furthermore, nano-Se is shown to be a potential source of Se for poultry/animal nutrition. However, there is an urgent need to address the questions related to nano-Se absorption, assimilation, and metabolism in animals/poultry before it finds its way to animal/poultry production as a feed supplement. This subject warrants further investigation.

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