



## MYTHS AND FACTS ABOUT THE EFFECTS OF NANO-SELENIUM IN FARM ANIMALS – MINI-REVIEW

Csilla Pelyhe<sup>[a]</sup> and Miklós Mézes<sup>[a]</sup>

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Selenium plays important roles in antioxidant defence systems, prevents cell damage and is necessary for growth, fertility, and immune system in farm animals. Recently, nano-elemental Se has attracted wide spread attention due to its high bioavailability and low toxicity, because nanometer particulates exhibit novel characteristics, such as great specific surface area, high surface activity, a lot of surface active centres, high catalytic efficiency and strong adsorbing ability and over and above the character of low toxicity of Se<sup>0</sup>. Nano-Se had similar or higher bioavailability and much less toxicity in broiler chicken and goat as compared to selenite, while in contrast some studies suggested that nano-Se was more toxic than selenite to Medaka fish. Feeding trials have proven that nano-Se supplementation had a positive impact on the growth, feed efficiency of the rumen, fertility and on the antioxidant status of the animals. However, the bioactivity of nano-Se depends on the size, while heat treatment has measurable effect on the size, structure, and bioactivity of selenium nanoparticles. This suggests that if extensive heat exposure is unavoidable during the feed processing, smaller sized selenium nanoparticles should be used. A basic understanding of the absorption, distribution, and clearance and the whole metabolic pathways and mechanisms of nano-Se should be modelled and next to this the understand of negative effects should be investigated and demonstrated in parallel with the novel, promising characteristics as well before using it in common in the animal nutrition.

\* Corresponding Authors

Fax: +36-28410804

E-Mail: Mezes.Miklos@mkk.szie.hu

[a] Department of Nutrition, Szent István University H-2103  
Gödöllő, Páter Károly u. 1., Hungary

Increasing the Se content of human foods by manipulating the source and level of Se supplementation to livestock is of interest to food scientists as well.<sup>7,8</sup>

### Introduction

The rapid development of nanotechnology holds great promises for application in medicinal and nutritional science, because nano-materials have been found to exhibit novel properties different to those at micro-scale and bulk materials.<sup>1</sup> According to the definition as given by the European Commission “nanomaterials are natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range”.<sup>2</sup>

Due to the advantages of size effect and high surface reactivity, nanoparticles have been already used in pharmaceutical applications to increase the bioavailability of drugs and targeting therapeutic agents to particular organs.<sup>3</sup>

Recently, nano-elemental Se has attracted wide spread attention due to its high bioavailability and relatively low toxicity, for instance LD<sub>50</sub> in mice 92.1 mg kg<sup>-1</sup> Se vs. 25.6 mg kg<sup>-1</sup> Se for nano-elemental Se and selenomethionine.<sup>4</sup> These differences based on that nanometer particles exhibit novel characteristics, such as great specific surface area, high surface activity, lot of surface active centres, high catalytic efficiency, and the character of low toxicity of elemental selenium.<sup>5</sup> It has been reported that selenium nanoparticles showed new characteristics of transport and uptake and exhibited higher absorption efficiencies as well.<sup>3,5</sup> Additionally nano-elemental Se has a size-dependent effect in directly scavenging free radicals *in vitro*.<sup>6</sup>

### Importance of selenium supplementation in feed for farm animals

Selenium has three levels of biological activities: (i) trace levels are required for normal growth and development; (ii) nutritional and supra-nutritional levels can be stored and homeostatic functions maintained; and (iii) toxic levels can result in deleterious effects.<sup>9</sup> Since the margin of safety of selenium is low, the potential toxic effects are important questions to be addressed when consider the role of selenium in dietary supplements.<sup>10</sup>

Dietary selenium is an essential trace element for animals and humans with a variety of biological functions.<sup>9,11</sup> It plays important roles in the regulation of thyroid hormone metabolism, cell growth and antioxidant defence systems thus, together with alpha-tocopherol prevents cells against oxidative stress damage<sup>12</sup>, also these compounds are necessary for growth, fertility, and immune system health in animals and humans.<sup>13,14</sup>

Animals consuming a diet low or deficient in selenium are prone to many problems, including white muscle disease in lambs and calves, calf pneumonia, infertility, and exudative diathesis in chickens and other animals.<sup>9</sup> Additionally, deficiency of selenium may cause increased formation of reactive oxygen species and/or decreased antioxidant defence, usually defined as oxidative stress.<sup>15</sup> Se-dependent glutathione peroxidases (GPx) are the most important peroxidases for the detoxification of hydroperoxides, and their activities have been widely used as biomarkers for assessing Se status in animals and humans.<sup>16</sup> Moreover, the determination of GPx activity has been shown to be an effective way to estimate the bioavailability of Se *in vivo*.<sup>17</sup>

The bioavailability of organic forms of Se (e.g. selenomethionine) is higher than that of inorganic forms of Se (e.g. sodium selenite) in monogastric animals<sup>18</sup>, and also in ruminants.<sup>19,20</sup> Currently, sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) is the main Se source used as a supplement in animal feeds, next to organic forms such as Se enriched yeast (selenomethionine) in the USA and in the EU.<sup>21,22</sup>

Subsequent studies report that nano-elemental Se possesses comparable efficiency to selenite, Se-methionine, and methylselenocysteine in upregulating selenoenzymes (e.g. GPx), and decreased acute toxicity.<sup>23,24,25</sup> Otherwise elemental selenium nanoparticles have higher bioavailability compared to other selenium compounds<sup>4</sup>, as it was proven in broiler chicken and goat compared with selenite.<sup>26,27</sup> These differences may be the result of the differences in lipophilic properties and metabolic pathways of the Se species.<sup>10</sup> Contrary to the above mentioned and generally accepted phenomenon that nano-Se is less toxic than inorganic or organic forms, it was showed that the toxicity of nano-Se (36 nm) is higher than that of selenite based on its lower  $\text{LC}_{50}$  in Medaka fish (*Oryzias latipes*).<sup>10</sup> Nano-Se caused 100% mortality at 3.2 mg L<sup>-1</sup> Se in fish, while selenite caused only 10% mortality at 2.0 mg L<sup>-1</sup> Se and 80% mortality even at 8.0 mg L<sup>-1</sup> Se. These authors found that nano-Se increase dramatically the selenium concentrations in fish liver (bio-concentration factors (BCFs) of nano-Se and selenite in fish liver were 320 and 25), while no increase in hepatic GPx activity was observed as compared to the controls, which suggests that seleno-enzymes in the Medaka liver were saturated before selenium supplementation. Moreover, nano-Se also caused more efficient accumulation of selenium in gills and muscles compared to selenite, with the differences ranging from two- to fourfold. The results revealed that nano-Se was more toxic than selenite for Medaka fish, and the value of the 48 h  $\text{LC}_{50}$  of nano-Se was fivefold lower than that of selenite. In conclusion, this experiment revealed that nano-Se exhibited more potent effects on disturbances to the antioxidant defence systems in Medaka fish compared to inorganic selenite, which is likely a consequence of hyper-accumulation of selenium.

### Absorption, tissue accumulation and excretion of selenium

The main route of absorption of inorganic selenium, such as selenite, is a passive diffusion from the intestine, and then 50–75% of total ingested Se is excreted in the urine.<sup>28,29</sup> This may be the reason of high selenium accumulation in kidneys<sup>26</sup>. In contrary to inorganic forms, organic Se, such as Se-methionine, is utilized in the intestinal wall by active transportation (e.g. by amino acid transport systems) and non-specifically incorporated into body proteins in place of methionine during protein synthesis, providing a means of reversible Se storage in organs and tissues.<sup>30</sup> Nanoparticles, such as nano-Se, are absorbed in the duodenum also by active transportation.<sup>31</sup> Otherwise an *in situ* study on the intestinal transport of <sup>75</sup>Se from ligated loop to body showed that the transfer of nano-Se from the intestinal lumen to the body was higher than that of selenite, while the intestinal retention of nano-Se was lower compared with selenite.<sup>32</sup>

In chicken the highest selenium concentrations was found in serum, liver and breast muscle, and it was increased as the dietary Se level increased (0.03 to 1.3 mg kg<sup>-1</sup> feed), but the

magnitude of increase was substantially greater when nano-Se was fed as compared to sodium selenite.<sup>32</sup> Animal studies have demonstrated that liver is the main target organ of Se toxicity<sup>33</sup> due to the consequence of hyper-accumulation of the absorbed selenium in liver and the fact that selenium-generated reactive oxygen species (ROS) formation are the major mechanisms for Se toxicity.<sup>34,35</sup> However, the addition of 1.20 mg kg<sup>-1</sup> Se from nano-Se did not cause signs of toxicity which suggest that the range between optimal and toxic dietary levels of nano-Se was wider than that of sodium selenite.<sup>32</sup> The possible cause of higher tolerance to selenium in form of nano-Se is its higher rate of retention in muscle, which may effectively reduce the available Se for inducing selenosis. This hypothesis supported by the study with intravenously administered [<sup>75</sup>Se]-nano-Se or [<sup>75</sup>Se]- $\text{Na}_2\text{SeO}_3$ , which showed that the percentages of nano-Se in the whole body was much higher than those of selenite.<sup>32</sup>

Bioavailability and toxicity of selenium nanoparticles depend on size. In a study the impact of heat treatment was investigated on the size, structure, and bioactivity of selenium nanoparticles at a nutritional (100 µg kg<sup>-1</sup> diet) and at supra-nutritional level (2000 µg kg<sup>-1</sup> diet) in mice.<sup>5</sup> Nanoparticles have an inherent tendency to grow into larger particles. In general, a smaller nanoparticle with a lower melting point grows uniformly, until it becomes stable at a specific heat treatment temperature through thermodynamic control.<sup>36</sup> It was showed that heat treatment causes selenium nanoparticles to aggregate into larger sizes and nanorods, leading to significantly reduced bioactivity in mice.<sup>5</sup> The thermo-stability of selenium nanoparticles is size-dependent, smaller selenium nanoparticles being more resistant than larger selenium nanoparticles to transformation into nanorods during heat treatment. It was found that, after a one-hour incubation of solution containing 80 nm selenium particles in a 90°C water bath, the nanoparticles aggregated into larger 110 nm particles and nanorods (290 nm × 70 nm), leading to significantly reduced bioavailability and resulted phase II enzyme induction. This result suggested that nano-Se causes oxygen free radical generation according to the hierarchical oxidative stress model<sup>37</sup>, which means that at the lowest level of oxidative stress the induction of antioxidant and protective responses is mediated by the transcription factor, Nrf2, which regulates the activation of the antioxidant response element in the promoters of phase II genes, such as glutathione-S-transferases.<sup>38</sup> When a solution containing 40 nm selenium nanoparticles was treated under the same conditions, the nanoparticles aggregated into larger 72 nm particles but did not transform into nanorods. Transformation into nanorods is probably responsible for the observed reduction in bioavailability. The above cited authors also found that unheated and heated 80 nm selenium nanoparticles both significantly increased hepatic and plasma GPx activity and hepatic selenium content accordingly a significant difference existed between the two selenium nanoparticle groups.<sup>5</sup> These results clearly demonstrate that the heat treatment significantly reduced bioavailability of the 80 nm selenium nanoparticles.

In an earlier study showed that oral administration of selenium nanoparticles to selenium-deficient mice at supra-nutritional selenium doses of 500 and 2000 µg kg<sup>-1</sup> for one week dose-dependently increased the activity of phase II enzyme, glutathione-S-transferase and tissue selenium levels.<sup>39</sup> Up-regulation of glutathione-S-transferase is important against oxidative stress-induced tissue or cellular

damage.<sup>40</sup> The unheated and heated 80 nm selenium nanoparticles both significantly increased hepatic glutathione-S-transferase activity and hepatic selenium accordingly a significant difference existed between the two selenium nanoparticle groups. These results suggest that temperature and duration of heat processing, as well as the original nanoparticle size should be carefully selected when a selenium nanoparticle solution is added to functional food or feed. There is also the strong implication that the use of smaller size selenium nanoparticles if extensive heat exposure is unavoidable.<sup>5</sup>

## Studies to demonstrate the effect of nano-Se supplementation in animal feed

### Performance parameters

A feeding trial with goats showed that the final body weight was increased significantly in bucks supplemented with selenium compared to the controls, and average daily gain in the group treated with nano-selenium and Se-yeast were significantly greater than the treated with sodium selenite or control bucks.<sup>41</sup> Another study with crucian carp (*Carassius carassius*) juveniles supports the positive effect of nano-Se on daily weight gain.<sup>42</sup> In a feeding trial with broiler chicken with addition of nano-Se (60 nm) showed a plateau of survival rate, average daily weight gain and feed to gain ratio when the Se concentration was 0.15–1.20 mg kg<sup>-1</sup> feed.<sup>32</sup>

### Rumen fermentation

A trial with cannulated sheep fed with ration containing 4 mg kg<sup>-1</sup> nano-Se of diet showed that nano-Se supplementation significantly decreased ruminal pH, ammonia concentration, molar proportion of propionate, and ratio of acetate to propionate were significantly decreased, but total volatile fatty acid concentration increased.<sup>43</sup> Furthermore total tract digestibility of dry matter, organic matter, crude protein, ether extract and fibre were also affected by feeding Se supplementation diets and with significantly higher values in nano-Se group compared to selenomethionine group. The same result was found by using 3 mg kg<sup>-1</sup> nano-Se diet.<sup>44</sup>

### Haematological parameters

Selenium, as the functional component of selenium-dependent GPx<sup>45</sup> protects the neutrophils and other blood components against peroxidative damage.<sup>46</sup> It was mentioned that selenium deficiency can increase oxygen free radicals in body tissues, the major negative effects of which are on the consistency of biological membranes and nano-Se with 1 mg kg<sup>-1</sup> of feed has a positive effect on the consistency of biological membranes and the performance of immunity cells.<sup>47</sup> In a study with sheep showed that nano-Se with 1 mg kg<sup>-1</sup> of feed has a positive effect against peroxidative damage in blood components.<sup>48</sup> The results revealed that lipid peroxidation was reduced after the 20<sup>th</sup> day of treatment, but the groups treated with sodium selenite only with more delay, which is shown the better antioxidant activity of selenium nanoparticles. Otherwise there were no significant differences between the packed cell volume and

red blood cell count among the groups. Nevertheless, the white blood cell count in nano-Se treated group showed a significant increase up to day 20 of treatment but it was not significant as compared to the controls. Also, there were significant increases of the neutrophil counts and significant decreases of the lymphocyte counts on day 10 in the nano-Se treated group as compared to inorganic selenium and control groups. Sum up, in sheep, selenium nanoparticles were found to be more bioactive than selenite.

### Male fertility

Many studies have shown that selenium is required for the maintenance of male fertility. Selenium deficiency in livestock leads to an altered and fragile midpiece with mitochondria being irregularly wrapped around the flagellum.<sup>49</sup> Oxidative stress is believed to be a major cause of sperm dysfunction, because sperm cells contain a high content of polyunsaturated fatty acids. The ratio of unsaturated to saturated fatty acids in small ruminant sperm membranes is also higher than in other species making the membranes quite vulnerable to the attack of the reactive oxygen species with loss of membrane integrity in the acrosomal region, impaired cell function and decreased motility of the sperm.<sup>50</sup>

A study with male goats showed that nano-Se (60–80 nm) supplementation with 0.3 mg kg<sup>-1</sup> of diet from weaning to sexual maturity has measurable effects on testicular microstructure, testicular spermatozoa ultramicroscopic structure, testicular GPx activity and semen quality in male goats.<sup>41</sup> Results showed that the testicular Se level and semen GPx activity increased significantly in the nano-Se supplemented group compared with control. The semen quality (volume, density, motility and pH) was not affected by selenium supplementation; however, ratio of abnormal sperm cells of control bucks was significantly higher than nano-Se supplemented bucks.

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