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Small particle, big impact: Nano-selenium as a next generation dietary supplement for broiler chickens: A review

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ABSTRACT

Trace minerals are essential for normal growth, health, reproduction and metabolism in poultry, yet the inorganic supplements traditionally used in feed are poorly absorbed, so much of what is fed is excreted and ends up as an environmental pollutant. Nanotechnology offers a way around this by reducing particle size and increasing the surface area available for absorption. Selenium (Se) is a key trace mineral and birds that do not receive enough of it show poorer growth, weaker immunity, reduced fertility and impaired antioxidant defence. Nano-selenium (Nano-Se) is increasingly viewed as a third generation Se source that the body uses more efficiently and that carries a much lower risk of toxicity than inorganic or organic selenium. This review brings together current evidence on selenium nutrition, metabolism, deficiency disorders and the comparative effects of inorganic, organic and nano-selenium on broiler performance, antioxidant status and immune function.

Keywords: Antioxidant, Broiler Chicken, Immunity, Nano-selenium, Nanotechnology

1. Introduction

Poultry production is one of the most rapidly expanding areas of animal science, yet many developing countries still fall well short of recommended per capita meat consumption. (FAO, 2011) [1] Feed costs make up anywhere from 65 to 80% of total expenditure in broiler and layer operations, so any improvement in how efficiently birds convert feed into body weight has an immediate economic impact. Within a nutritionally complete diet, trace minerals play a central role in the enzymatic and biochemical processes that govern growth rate, egg production and feed utilisation. (Leeson *et al.*, 2001) [2]

Selenium (Se) is a non-metallic trace element (atomic number 34; atomic weight 78.96) belonging to Group VI of the periodic table. It was discovered by Jöns Jacob Berzelius in 1817 and for most of the following century it was regarded primarily as a poison. Its importance as a nutrient was only established in 1957, when Schwarz and Foltz showed that Se protected rats against dietary liver necrosis. (Schwarz *et al.*, 1957) [3] A great deal of subsequent work confirmed that Se acts as a key antioxidant through the enzyme glutathione peroxidase (GPx), helps regulate thyroid hormone levels via deiodinase enzymes, supports immune function and is necessary for normal reproduction. In poultry and other farm animals, Se deficiency leads to measurable falls in both productive and reproductive performance. (Surai, 2006) [4]

Sodium selenite and sodium selenate, the inorganic Se sources that have traditionally been added to poultry feed are not particularly well absorbed and can only be included at low levels because the gap between a useful dose and a toxic one is narrow. Organic forms such as selenomethionine and selenium yeast are better absorbed. More recently, Nano-Se has emerged as a third-generation source: it has a higher surface activity, is absorbed more readily and is substantially less toxic at equivalent dietary concentrations. (Li *et al.*, 2008) [5] This review examines the published literature on the role of selenium in broiler nutrition, with particular attention to the potential advantages of the nano form.

2. Nanotechnology

The word "nano" comes from the Greek for "dwarf" and refers to one billionth of a metre. Nanotechnology covers the design, characterization and practical use of materials and devices at the 1–100 nm scale. At this size, the huge increase in surface area to volume ratio gives particles properties that are simply not seen in larger, bulk versions of the same material. (Feng *et al.*, 2009) [6]

The term was first used by Taniguchi, 1974 [7] to describe precision machining using electron, ion and laser beams. (Taniguchi, 1974) [7] Since then, nanotechnology has been described as a new industrial revolution,

(Schmidt, 2009) [8] with wide ranging applications in food production, processing, packaging, traceability and safety. (Otlés *et al.*, 2008) [9] In the livestock and poultry sector, nanoscale mineral particles are of particular interest because the greatly enlarged reactive surface area helps the body absorb and retain them more efficiently than conventional supplemental forms.

3. Selenium and its role in poultry nutrition

Selenium carries out most of its biological work through selenoproteins, proteins that contain the amino acid selenocysteine at their active site. More than 25 selenoproteins have been identified in mammals. Among the most important are the glutathione peroxidase (GPx) family, the thioredoxin reductases and the iodothyronine deiodinases. GPx enzymes break down hydrogen peroxide and organic hydroperoxides, making them a frontline defence against oxidative damage, while the deiodinases control thyroid hormone levels by converting the inactive hormone thyroxine (T4) into the active form tri-iodothyronine (T3). (Surai, 2006; Rotruck *et al.*, 1973) [4, 10]

In commercial broiler flocks, Se touches virtually every aspect of productive output. When dietary Se is adequate, tissues that are rich in polyunsaturated fatty acids including sperm and embryonic membranes are protected against oxidative damage, which keeps fertility and hatchability at acceptable levels. Se also helps to regulate both innate and adaptive immunity, contributes to normal feather formation and ensures that thyroid hormones can drive efficient nutrient metabolism. (Choct *et al.*, 2004) [11]

Inorganic Se forms (sodium selenite, sodium selenate) are the most widely used supplements, but their transfer from feed into body tissues is relatively poor and their cytotoxicity means they cannot be added in large amounts. In practice, GPx activity in plasma, liver, or erythrocytes is used as the standard marker of Se nutritional status in poultry and as the main yardstick for comparing different supplementation strategies. (Beck *et al.*, 2007; Neve, 1995) [12, 13]

4. Selenium deficiency in poultry

When Se is deficient, particularly in combination with low vitamin E, a recognizable set of clinical problems emerges in poultry. Exudative diathesis, which presents as subcutaneous fluid accumulation due to leaky capillary walls, was one of the earliest conditions found to respond to Se supplementation. (Bartholomew *et al.*, 1998) [14] Nutritional pancreatic atrophy (NPA) produces fibrosis and degeneration of the exocrine pancreas, causing poor digestion, slow growth and abnormal feathering. (Thompson *et al.*, 1969) [15] Encephalomalacia, caused by unchecked lipid peroxidation in the cerebellum, is the most serious neurological outcome of combined Se and vitamin E deficiency. (Combs *et al.*, 1991) [16]

In laying hens and broiler breeders, Se deficiency depresses egg production, fertility and hatchability and increases embryonic death. Latshaw and Osman showed in 1974 [17] that low dietary Se reduced reproductive performance and that this could be fully corrected by supplementation. (Latshaw *et al.*, 1974) [17] Eggs from severely Se-deficient hens had infertility rates of 12.6%, embryonic mortality of 29% and hatchability as low as 15%. (Latshaw *et al.*, 1977) [18] Immune competences is also impaired, with birds showing weaker responses to antigens and greater vulnerability to infection. (Combs *et al.*, 1986) [19]

5. Selenium metabolism in poultry

Most of the Se in poultry diets arrives via plant based ingredients. Plants take up inorganic selenate and selenite from the soil and convert them into organic forms, principally selenomethionine (SeMet), which gets incorporated into plant proteins wherever methionine would normally go. The Se content of feed ingredients therefore depends heavily on the Se concentration of the soil from which the crops were grown, and this varies considerably from one region to another.

When inorganic Se is consumed, selenate is first reduced to selenite by enzymes and selenite is then reduced non-enzymatically by glutathione to form seleno-diglutathione. Under low oxygen conditions, glutathione reductase converts this intermediate to hydrogen selenide (HSe), which is the central metabolite from which all other Se pathways branch off. (Ganter, 1966) [20] From there, Se can be methylated to volatile compounds excreted in breath and urine, bound to Se carrying proteins, or used by selenophosphate synthetase to insert selenocysteine into growing selenoproteins via a specialised mechanism involving the UGA codon. (Sunde, 1997) [21]

Organic Se follows a different route. Dietary SeMet is taken up efficiently and incorporated into body proteins in place of methionine, building up a substantial and slowly released Se reserve in skeletal muscle. As proteins turn over in the normal course of metabolism, this SeMet is released and eventually converted through the trans sulphuration pathway to selenocysteine, which is either used for selenoprotein synthesis or broken down by selenocysteine lyase to elemental Se and then to selenide. (Hoffman *et al.*, 1970; Esaki *et al.*, 1982) [22, 23] This ability to store Se in muscle and release it gradually explains why organic Se supplements particularly selenomethionine enriched yeast achieve higher Se levels in tissues and sustain GPx activity more consistently than equivalent doses of inorganic Se.

6. Characterisation of different forms of selenium

6.1 Inorganic selenium

Sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) are the most commonly used inorganic Se supplements in commercial poultry feeds. Compared with other forms, they are not particularly bioavailable and the safety margin between a nutritionally effective dose and a toxic one is narrow; roughly five to tenfold. This restricts how much can be included in feed and limits Se deposition in eggs and muscle.

6.2 Organic selenium

Se-enriched yeast is produced by growing *Saccharomyces cerevisiae* in a medium that is rich in selenium but restricted in sulphur, so the yeast incorporates Se mainly as selenomethionine. Regulatory bodies in the European Union and the United States permit Se yeast at up to 0.3 ppm in complete feeds. Organic Se sources are consistently better absorbed and transfer Se more effectively into eggs and muscle than inorganic forms, primarily because SeMet is taken up by the same intestinal transporters as methionine and stored freely in muscle protein. (Utterback *et al.*, 2005; Payne *et al.*, 2005) [24, 25]

6.3 Nano-selenium

Elemental Se at the nanoscale; typically 20–100 nm, appearing as a red amorphous solid, behaves quite differently from both inorganic and organic Se. The enormous specific surface area relative to bulk elemental Se means it interacts far more readily with intestinal epithelial transporters and is converted more rapidly inside cells to selenide, the obligate starting point for selenoprotein production. Cai *et al.* reported that Nano-Se supplementation at 0.3–2.0 mg/kg improved broiler growth, meat quality and oxidative resistance without causing any adverse effects on blood or biochemical parameters. (Cai *et al.*, 2012) [26] There is also evidence that Nano-Se switches on the genes encoding selenoproteins more effectively than equivalent doses of sodium selenite, probably because the nanoparticle surface acts as a sustained intracellular Se reservoir that matches the rate at which selenoproteins are synthesised. (Li *et al.*, 2008) [27]

7. Effect of different selenium sources on body weight performance

Selenium influences how fast broilers grow through several interacting mechanisms. By keeping the enzyme 5'-deiodinase active in the liver and other tissues, adequate Se ensures that circulating levels of the active thyroid hormone T3 remain normal. T3 sets the basal metabolic rate and drives the hormonal signalling that promotes muscle

growth. When Se is deficient, T3 availability falls and growth slows, quite independently of how much the bird is eating. (Mikulski *et al.*, 2009) [28] Se-sufficient birds also develop better feathering; good plumage reduces the amount of energy needed to maintain body temperature, leaving more nutrients available for weight gain. (Choct *et al.*, 2004) [29]

A large number of feeding trials; using inorganic, organic and nano forms of Se; have confirmed that supplementation improves live weight gain compared with Se-deficient controls. (Thompson *et al.*, 1969; Cantor *et al.*, 1982) [30, 31] Organic Se generally gives a slightly better growth response than sodium selenite at the same dietary concentration, largely because of the sustained muscle Se reserve, better T3 status and additional immune benefits. (Surai, 2006) [4] The size of the growth response varies with the basal Se content of the diet, the genetic line of bird, health status and the environmental conditions under which the study was conducted.

8. Effect of different selenium sources on feed intake and feed efficiency

The effect of Se supplementation on how much birds eat voluntarily is generally small and inconsistent between studies. Several trials found no meaningful difference in feed consumption between birds given graded levels of inorganic or organic Se. (Paton *et al.*, 2002; Skrivan *et al.*, 2008) [32, 33] Swain *et al.* did, however, find the best daily weight gain and the most favourable feed conversion ratio (FCR) in broilers receiving 0.50 mg Se/kg combined with 300 IU vitamin E/kg, suggesting these two antioxidant nutrients work together in a way that is greater than the sum of their individual effects. (Swain *et al.*, 2000) [34]

The improvements in FCR seen with organic and nano-Se supplementation are mainly attributed to better feathering (which lowers maintenance energy costs), optimised nutrient partitioning toward lean tissue under adequate thyroid hormone drive, and reduced oxidative damage to the gut wall, which makes the intestine better at absorbing nutrients. (Hoffmann, 2007; Ozkan *et al.*, 2007) [35, 36] Zhou and Wang showed that dietary Nano-Se at 0.3 mg/kg significantly improved FCR and GPx activity in Guangxi Yellow chickens compared with the same dose of inorganic Se. (Zhou *et al.*, 2011) [37] Differences in results between published studies likely reflect variation in bird genetics, basal diet composition, ambient temperature and the housing conditions at each site.

9. Effect of different selenium sources on antioxidant properties

9.1 Oxidative stress in poultry

Commercial broiler production puts birds under several types of stress at once; high stocking density, heat, pathogen pressure and the physiological demands of fast growth; all of which push the body to produce excess reactive oxygen species (ROS). When ROS generation outpaces the body's ability to neutralise them, lipid peroxidation damages the fatty acid chains in cell membranes, oxidised proteins lose their function and breaks in DNA strands disrupt cell division. The resulting oxidative stress drives down feed efficiency, lowers meat quality and reduces flock survival. (Jaeschke, 1995) [38]

9.2 Key selenium-dependent antioxidant enzymes

The GPx family; particularly GPx-1 in the cytoplasm, GPx-4 associated with membrane phospholipids, and GPx-3 in plasma are the main Se-dependent antioxidants in poultry tissues. At the catalytic centre of each enzyme, a selenocysteine residue drives the two electron reduction of harmful hydroperoxides to safe alcohols, using glutathione as the electron donor. Se availability is the primary factor controlling how much GPx the body produces; even modest Se restriction quickly reduces GPx activity in the liver, blood plasma and red blood cells. (Maiorino *et al.*, 1995) [39]

Superoxide dismutase (SOD) converts the superoxide radical ($O_2^{\cdot -}$) to hydrogen peroxide and molecular oxygen, providing early protec-

tion against ROS injury. (Kappus, 1985) [40] Catalase (CAT) then breaks down hydrogen peroxide into water and oxygen in the liver, kidneys and red blood cells. Taken together, GPx, SOD and CAT form a stepwise antioxidant cascade in which Se availability directly determines the efficiency of the first and rate limiting step.

Cai *et al.* showed that Nano-Se supplementation at 0.3–2.0 mg/kg raised GPx activity and reduced malondialdehyde (MDA) concentrations in broiler breast muscle in a dose dependent manner. At equivalent inclusion rates, the nano form produced better antioxidant indices than sodium selenite, consistent with its superior bioavailability. (Cai *et al.*, 2012) [26]

10. Effect of different selenium sources on immunity

10.1 Cell-mediated immunity

Selenium supports T-lymphocyte function through several pathways: it promotes the incorporation of selenocysteine into selenoproteins within activated T cells; it enhances expression of the interleukin-2 (IL-2) receptor, amplifying clonal expansion; and thioredoxin reductase, a selenoprotein, maintains the reducing conditions that T cells need to become activated and to divide. (Brown *et al.*, 2001) [41] When Se is deficient, the oxidative burst of heterophils and macrophages weakens, natural killer cell activity falls and T-cell proliferative responses to mitogens are blunted. (Arthur *et al.*, 2003; Kiremidjian-Schumacher *et al.*, 1992) [42, 43]

10.2 Humoral immune response

Adequate Se is associated with better antibody responses after vaccination and natural antigen exposure. Several studies in broilers and layers reported higher haemagglutination-inhibition (HI) titres against Newcastle Disease Virus and stronger antibody responses against sheep red blood cells in Se-supplemented compared with Se-deficient birds. (Hegazy *et al.*, 2000; Larsen *et al.*, 1997) [44, 45] Arshad *et al.* showed specifically that dietary Se supplementation significantly raised post-vaccination antibody titres against Infectious Bursal Disease Virus (IBDV) in broiler chickens, a finding with direct practical value for improving vaccine protection under field conditions. (Arshad *et al.*, 2005) [46] Leng *et al.* further established that organic Se sources boost the humoral immune response in poultry more effectively than equivalent concentrations of inorganic Se, most likely because of greater bioavailability and consequently higher selenoprotein levels in lymphoid tissues. (Leng *et al.*, 2003) [47]

11. Conclusion

Selenium is an essential trace element whose adequate supply in the diet is critical for antioxidant defence, thyroid hormone metabolism, immune function and reproductive performance in broiler chickens. Of the three supplemental forms available; inorganic, organic, and nano-selenium; Nano-Se shows the most consistent pattern of superior bioavailability and a wider safety margin at equivalent dietary concentrations. Supplementing diets with Nano-Se improves live weight gain, feed conversion ratio and GPx activity, reduces oxidative damage markers in muscle and strengthens both cell-mediated and humoral immune responses. The weight of current evidence supports Nano-Se as a viable, highly bioavailable and lower-risk alternative to conventional selenium supplements in commercial broiler production. Further dose titration studies and multi-site trials under different climatic conditions are needed to determine the optimum Nano-Se inclusion levels for the range of production systems and genetic lines in use today.

12. Conflict of interest

The authors declare that they have no conflict of interest.

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