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# Chapter

# Essential Nanominerals and Other Nanomaterials in Poultry Nutrition and Production

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# Abstract

Poultry production, health and wellbeing are highly dependent upon formulation of balanced rations in terms of energy, protein, and micronutrients (vitamins and minerals). Among all, minerals are required in fewer quantities, but they are very important to maintain the productivity in poultry. Minerals present in the feeds are less bioavailable and additional supplementation is obligatory to meet the physiological demands of poultry. Conventionally, minerals are supplemented as inorganic salts, which are less absorbed and, thus, a major proportion is excreted to the surroundings creating environment issues. Nano-minerals and organic mineral chelates are other alternative to be used as livestock and poultry feed supplements. Though organic minerals are more bioavailable than inorganic salts, their high cost limits its use. In contrast, nano-minerals are relatively easy to synthesize at a lower cost. Nano-minerals are of the size from 1–100 nm and due to such small size, there is an enormous increase in surface area and thus their biological responses. The biological response studies have signified better retention of nano-minerals as compared to inorganic salts, and consequently leached less to the environment preventing possible pollution. Apart from these, nano-minerals have been shown to enhance growth, egg production and quality, immune-modulation and antioxidant status, and at the same time economize the production by reducing the supplemental dose of minerals and improving the feed conversion ratio. Some nano-minerals and other nanoparticles have strong antimicrobial effects, which have been shown to reduce pathogenic microorganisms in the gut. Nano-minerals seem to be less toxic than conventional mineral sources. Though less, few studies have indicated toxic effects of nano-mineral supplementation at higher dose of application, which should be validated by more programmed studies. Nanotechnology in poultry production system is still in its budding stage and more detailed studies are warranted to validate, establish and search for new effects of nano-minerals as they sometimes produce effects beyond expectation. This review highlights the biological responses of nanominerals on poultry production performance, quality of meat and eggs, tissue retention, immunity, antioxidant activity and antimicrobial actions compared with their conventional mineral sources.

**Keywords:** antimicrobial, health, nano-mineral, performance, poultry, quality, retention, toxicity

# 1. Introduction

Mineral nutrition is an indispensible part of animal feeding system which ensures optimum health, production, and reproduction in animals and birds. Even though, required in small quantities as compared to other nutrients such as energy and protein, their deficiency and imbalances are promptly reflected in the changes of animal wellbeing and their production. Sometimes, this may also cost the animals with their lives. They are essential for maintaining the normal health and productions; whereas in some cases additional supplementation could yield better growth and egg production. The significance of mineral nutrition is well documented and still new projects are undertaken to understand, explore better aspects and validate newer postulates associated in the field of mineral nutrition.

Conventionally, minerals are used in the diets through their inorganic salts, but low bioavailability of inorganic mineral salts necessitates using at higher doses in order to meet the animal requirements, which indirectly creates more pollution with minerals [1]. Recently, nano-sized minerals are considered to have greater bioavailability in animals and birds due to increased surface area, which tend to produce better desirable responses [2]. This chapter discusses synthesis of different nano-minerals, their mechanism of action, poultry performance, tissue retention, immunity, antioxidant activity and antimicrobial actions compared with their conventional mineral sources.

# 2. Importance of mineral nutrition in biological system

Minerals are vital for all biochemical functions in the body along with providing structural supports, electrolyte balance and homeostasis. The requirements of calcium (Ca) and phosphorus (P) in animals and poultry are comparatively greater than other minerals. They are mainly needed for bone development [3]. Zinc (Zn) is essential for several physiological and biochemical processes such as normal growth, reproduction, wound healing, ossification, DNA synthesis, cell division and gene expression, photochemical processes of vision, and augmenting the immune system of the body through lymphocyte replication and antibody production [1–4]. Selenium (Se) is essential for optimum animal production, fertility, and disease prevention [3]. However, role of Se in intra- and extra-cellular antioxidant systems is vividly recognized [5], which, as a component of glutathione peroxidase (GPx) neutralizes hydrogen and lipid hydroperoxide and thus maintains membrane integrity and guards from oxidative damage of lipid membranes [1]. Copper (Cu) is essential for normal growth, bone development, immune response, foetal development, nerve functioning, and in antioxidant system as a part or a cofactor of several enzymes [1]. Manganese (Mn) is an essential trace mineral necessary for optimum antioxidant, immune system as well as a component on several important enzymes [2]. Likewise, iron (Fe) is needed for synthesis of hemoglobin, which transports oxygen in the body and myoglobin, and is also associated with enzymes, e.g., peroxidases, hydroxylases, and catalase. Chromium (Cr) is a component of glucose tolerance factor and is essential for maintaining immune and antioxidant function and metabolism of lipids and proteins [6]. Combining all the effects together, minerals are associated with all the physiological functions in the body either involved directly or indirectly. Hence, a diet balanced in all the minerals is always a matter to maximize the productivity and health of the animals.

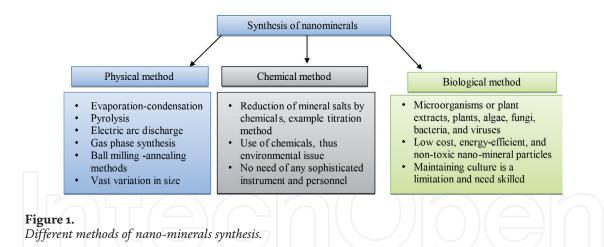
# 3. Sources of minerals

The minerals are present in all the food and feed sources as an integral part, but the amount required to support the productivity is not met through the feed resources. Added to this, the mineral component from plants are less absorbed and retained in the body as they form complex compounds with other components. For an instance, plant ingredients in the diets contain large amounts of unavailable P as phytates, which accounts almost 60–80% of total P and are not absorbed by the birds due to insufficiency of phytase enzyme [7]. Again, the bioavailability of minerals from traditional inorganic sources is relatively less for many minerals, while the requirements for high producing animals and birds are very high [8]. This necessitates the addition of minerals in the diets from extraneous sources [1, 9, 10], which gives the concepts of minerals as feed supplements in animal and poultry rearing. Minerals are generally supplemented in higher concentrations than their actual needs at cellular levels when inorganic supplements are added due to their poor bioavailability [11] along with chemical antagonism and interactions with other nutrients [12]. Conventionally, minerals are supplemented in the diets in their inorganic salts — oxides, sulphates, or carbonates — for instance, Zn oxide, Zn sulphate, sodium selenite, Ca carbonate, and dicalcium phosphate (DCP). The low bioavailable inorganic mineral salts supplemented at higher doses in order to meet the animal requirements, indirectly creates more pollution with minerals [1, 9, 10]. This issues needs to be addressed and better bioavailable mineral sources are a thrust of mineral studies for many decades. Many organic chelated minerals have been tried to fill the gap and reports indicated mixed responses considering their bioavailability, cost effectiveness and biological responses. Organic minerals as proteinate supplemented retained better in poultry signified better bioavailability as compared to their inorganic counterparts [13, 14]. Organic mineral supplementation has shown varied type of response in layers. For example, Rajendran et al. [15] reported improvement in laying percentage of birds, whereas Soni et al. [16] did not observed any effect on egg production by feeding organic minerals. In spite of better bioavailability of organic minerals over their inorganic counterparts, these sources are less used due to their higher cost [17]. This necessitates the urgent requirement for better bioavailable sources to be used particularly in poultry production to save guard the environment without affecting the animal or bird productivity at a cost effective manner.

Recently, nano-sized minerals are considered as a potential alternate to fill the gap and they have been tried and tested in many ways to validate their better bioavailability in diversified animals and birds. Nanotechnology confers the materials with particle size in nanometer (nm) range (<100 nm) at least in one direction, and by virtue of the nano-sized particle (NP), their structures exhibit significantly novel physical, chemical, and biological properties and functionality [18]. Due to their small size, the surface area increases many folds and thus they tend to produce many desirable responses [2]. The altered chemical and physical properties of NP could potentially modify the biological responses compared to its bulk materials [2, 19]. Studies have been carried out across the globe to unveil more beneficial effects as a feed additive in animals and birds, but still nanotechnology is in its infancy in the animal husbandry sector. In this chapter, we have tried to compile the various effects of the nano-minerals and other nano-materials in poultry.

### 4. Nano-minerals: synthesis

Nanotechnology deals with research and development related to nano-sized materials, and are specifically focused at understanding of measurement and



manipulation of matters at the nanoscales. Use of NP is gaining importance in diversified disciplines starting from medicine, environment, food, electronics, pharmaceutical applications, biotechnology, agriculture, and animal science [2]. Nano-minerals are specially synthesized mineral particles with its particle size ranging from 1 to 100 nm [20]. Like NP, nano-minerals possess higher physical activity and chemical neutrality, which may be a reason for efficient absorption in the animal system [21] and are reported to be stable under high temperature and pressure [22] as well. Nano-minerals as feed supplement can increase the feed efficiency, diminishing feed cost by reducing the supplemental doses, and simultaneously intensifying the yield and value of animal products by virtue of their superior bioavailability [1, 23, 24]. For example, nanominerals, due to their smaller size, were reported to be easily taken up by the gastrointestinal tract and efficiently utilized in vivo, and hence were more effective than the larger sized zinc oxide (ZnO) at lower doses [20]. Moreover, nanominerals exhibit lesser adverse effects as compared to their conventional counterparts. For instance, Reddy et al. [25] reported that nano-ZnO had less adverse effect on human cells. Nanominerals can cross the small intestine and further distribute into the blood, brain, and other different organs [26]. The functional properties of nanominerals, such as chemical, catalytic or biological effects, are highly influenced by their particle size, shape, composition, crystalline structure, surface ions, and morphology [27–29]. Nano minerals can be synthesized by physical, chemical or biological methods (**Figure 1**) [1, 19]. In physical method, physical forces are used to break down the larger sized materials to nanoscale, whereas in chemical method, reducing agents are used to reduce the particle size. Nanomaterials produced from physical method have wide range of particle size, but chemical method produce tentatively uniform particle size [19]. In biological method, also called green synthesis, different plant products or cultures are used for reducing the size of the intended materials. This method is free from use of corrosive chemicals which is the main constraint in chemical synthesis of NP. However, maintaining the culture needs technical expertise and is considered as a limitation in this method. Considering all points and methods, for use in livestock and poultry feeding, chemical method seems preferred as they are cheap, easy to produce and do not require any special instrument and expertise [19].

# 5. Mechanism of action of nanominerals

Nanoparticles are quite different in physical properties from bulk materials, contributing to wide range of new applications. Due to the much-reduced particle size they exhibit novel and improved physical, chemical, and biological activity that do not necessarily resemble the bulk mineral counterpart, and thus numerous modes

of action are postulated by different workers. We have tried to compile the available resources keeping poultry nutrition in view. As such, further studies are warranted to establish or abolish any mechanism of action postulated till date.

Possibly the increased surface area of NP facilitates better interaction in biological interface and their increased retention time in the gut, reduced influence of intestinal clearance mechanisms and effective delivery of functional compounds to target sites result in better bioavailability and functionality [30]. By virtue of their small size, uptake by the gastrointestinal epithelium is much easier [20]. Uptake of NP through mucosal layer is dependent upon the charge on their surface and pH of adjacent environment. Changes in pH alter the surface charge and thus lead to agglomeration and change in size [31, 32]. For example, cationic NP was reported to be trapped within the glycosylated areas of mucin, whereas the diffusion of carboxylated anionic microparticles through the epithelial surface was better [33]. Nanoparticles are either absorbed through epithelial villi into the circulation and are subsequently transported to the liver and spleen [20, 34] or through M-cells in the Peyer's patches crossing the enterocytes and pass into the hepatic circulation [35]. Due to the smaller pore size (0.6 to 5 nm) of tight junctions, paracellular transport of NP is usually limited under normal physiological conditions [36]. Trace element NP may decrease mineral antagonisms in the intestine leading to enhanced absorption and utilization, thereby lowering their excretion into the environment. They are chiefly transported by transcellular mechanism. After absorption, their dispersion, breakdown, and discharge are related to their dissolvability, charge, and size. Nano-minerals have the potential to enter the blood, brain, lung, heart, kidney, spleen, liver, intestine and stomach after crossing the small intestine [26]. But their uptake rate in intestinal epithelia and other body tissues substantially differs [37]. The particle sizes less than 300 nm can reach to the blood circulation, whereas particles smaller than 100 nm can penetrate various tissues and organs [38].

# 6. Mineral absorption and metabolism

The amount of mineral absorbed and retained is termed as bio-availability, and this can be reflected by improved performance of animals or birds. Better bioavailability is indicated by more amount of mineral deposits in the organ, serum, and also better biological responses, and is affected by factors that influence absorption such as concentration, chemical forms, transport pathway, nutrient-nutrient interactions and excretory losses. Reports suggest that the bioavailability of inorganic salts is less, which results in high excretion of minerals into the environment through urine and feces [39]. Considering the other potential replacement of inorganic salts, organic and nano-minerals have provided encouraging biological effects when fed to animals and birds [1, 8, 21, 40, 41] with certain limitations.

Of the different mechanisms of transportation through intestinal epithelium, paracellular transport involves passage of substances across the epithelium through the intercellular spaces whereas transcellular transport involves passage of substances through the cells [42, 43]. Paracellular transport does not include any transporter or energy expenditure for transport and the absorption occurs along the concentration gradient, thus is not very efficient [42]. Tight junctions act as gate-keeper of paracellular transport and they exclude entry of macromolecules [42, 43]. Transcellular absorption involves either diffusion across concentration gradient or active carrier mediated transportation utilizing energy or through endocytosis [42]. Intestinal absorption can be improved by altering paracellular and transcellular transport. Compared with CuSO4 and CuO microparticles, CuO NP are believed to be rapidly transported into cells, and subsequently interact with the Cu transport

proteins [44], with a non-antagonism of CuO NP with Zn. Na et al. [45] suggested the possibility that CuO NP are absorbed through a different pathway that other Cu sources use [1].

# 7. Nano-mineral supplementation and mineral retention

Liver handles most of the absorbed nutrients and regulates their release into blood circulation for further distribution in different tissues or excretion. Hence, increased concentration in liver is a suitable indicator of retention status. The minerals supplemented in their NP forms increase their bioavailability and utilization efficiency [46]. Minerals supplemented in their nano-forms retained better in in vivo studies as compared to their inorganic salts [41, 47, 48]. Patra and Lalhriatpuii [1] extensively reviewed the retention of nano mineral supplementation in poultry and suggested that Ca, P, Zn, Cu, Se, Mn and Fe in their nanoforms are retained better than their inorganic counterpart. Owing to better bioavailablity, Ca and P supplementation as nanominerals reduces the quantity of supplementation, thus making the ration economic and environment friendly [1]. Sohair et al. [49] reported that the use of hydroxyapatite NP is economically efficient as compared to the control diets. Nano-Zn in lower dose could be a good substitution in mineral premix instead of ZnO, which tend to improve carcass characteristics and oxidative stability of chicken meat [50]. Hu et al. [51] studied the selenium retention from nano-Se and selenite origins in chickens by the intravenous or oral administration of the radio labeled <sup>75</sup>Se and the in vivo ligated intestinal loop procedure, and reported higher nano-Se retention in the whole body and liver tissue compared to that of selenite, and intestinal transport of Se through ligated intestinal lumen loop to body was higher than that of selenite. Retention of Se is found to be influenced by the dietary Se source and concentration of Se supplemented as well. Reports suggests nano-Se supplementation to Guangxi Yellow broilers diet improved hepatic and muscle Se contents in a dose dependent manner up to a supplemental dose of 0.3 mg/kg [52], suggesting a relationship between nano-Se metabolism and liver function. Similar responses were also reported by Meng et al. [53] and Mohapatra et al. [54], where they obtained higher Se deposition efficiency in nano-Se supplementation groups than sodium selenite group, which also suggests better retention of nano-Se than that of sodium selenite as suggested by Zhang et al. [55]. Radwan et al. [56] observed higher Se content in eggs by nano-Se supplementation as compared to sodium selenite, most probably due to the faster transfer of nano-Se into the egg. A linear and quadratic increase in liver and muscle Se in a proportionally to the dietary nano-Se level, with a the peak value at 2.0 mg/kg of dietary nano-Se was reported; however, considering meat quality, immune function, oxidation resistance, 0.3 to 0.5 mg/kg was reported to be the optimum level of supplementation of nano-Se for broilers [57]. Supplemental nano-Cr picolinate at 0.5 and 3 mg/ kg of Cr increased Cr and Ca concentration in the liver and egg, and improved Zn and Mn retention in layer chickens [58]. Nano-Cr added at 0.4 mg/kg feed was found to increase the retention of Cr, Ca, P, Zn, and Fe in layers, increased the Cr and Zn concentration in plasma, liver, and eggshell; zinc in egg yolk; Ca in the liver and eggshell [59]. However, no increase in Cr content in the eggs and blood of Japanese quails was reported due to dietary addition of 0.2 to 0.8 mg/kg of nano-Cr [60]. Jankowski et al. [61] reported no effect of reducing Mn from 100 to 50 or 10 mg/kg either from NP-Mn2O3 or MnO on the growth performance or oxidation process in liver and breast muscles and increased Mn accumulation and reduced Zn and Cu accumulation in the liver, breast muscle and skin but increased intestinal absorption of Zn. Nano MnSO<sub>4</sub> supplementation resulted in improvement of tibia

bone characteristics such as tibia length, tibia volume, tibia breaking strength, tibia diameter and bone weight [1]. Intramuscular injection of Fe-NP at 2 mg/ kg BW improved body weight gain, hematological traits, and tissue retention of Fe in broiler chickens [62]. The enrichment of the food with nano-Fe improved Fe concentration in the body (by 5.3%) and erythrocyte in blood [63]. Cysteine-coated Fe3O4 NP at 1.2 mg/kg diet recorded similar weight gain, feed efficiency, hematological and biochemical parameter as that of 120 mg/kg of FeSO4 in a quail diet [64]. Overall, the above observations suggest that different mineral NP usually increased retention of minerals in tissues, eggs and meat even at the lower doses compared with their conventional sources of minerals.

# 8. Growth performance and meat quality

Most of the studies on poultry has emphasized on growth promoting effect as well as mineral retention due to supplementation of nanoforms of minerals (**Figure 2**). Studies have shown growth promoting effects by feeding nano-Zn [48, 50, 65, 66], nano-Se [52, 54, 67], nano-Ca [49, 68, 69] and nano-Ag [70]. Mohammadi et al. [71] observed improved growth performance in broilers supplemented with nano-Zn-methionine and nano-Zn-max at 80 mg/kg of diet; however, dietary nano-Zn sulphate reduced growth performance in broilers. Nano-Ag supplementation at 4 mg/kg caused an improved body weight gain and best feed conversion ratio in broiler [70]. Silver acting as an antimicrobial agent on intestinal harmful bacteria may improve gut health leading to better nutrient absorption, which was manifested by improved weight gain, feed intake and feed conversion ratio of broilers fed diets containing Ag NP [72]. Nano-Ca phosphate at 50% level of recommended supplementation resulted in improved body weight gain without altering feed conversion ratio, carcass characteristics and biochemical parameters similar to the 100% DCP supplemented group [73].

Supplementation of DCP NP has been reported to show better feed conversion ratio and body weight gain in poultry at 50% levels [68] and at 1.75, 1.31, and 0.88% levels [69] when compared to the control groups fed with larger DCP particles. Similar to DCP NP, hydroxyapatite NP also increased growth rate and feed intake in broilers at 2 to10% supplementation [49] without affecting the digestibility coefficient of other nutrients. However, Sohair et al. [49] observed that supplementation of 0.12% of calcium phosphate-NP instead of the conventional DCP at 2% resulted in better body weight gain, feed efficiency and economic efficiency

<u>Growth</u> Improvement in body weight gain, feed efficiency

Egg production -Improves egg production, egg weight, hatching percent, egg quality parameters

<u>Antimicrobial activity</u> -Decreases microbial load -Improves quality of product



Haematology and blood biochemistry -Improves antioxidant enzymes, hormones related to growth and reproduction, and immunity related cells like lymphocytes, globulin , and albumin. Immunity -Improves cell mediated- and humoral immunity

<u>Meat production</u> -Improvement in meat quality -Improvement in keeping quality

<u>Miscellaneous</u> -Retained more efficiently -Less excretion -Reduce environment pollution in broiler chickens. Mishra et al. [48] fed layer birds with extremely low levels of nano-Zn (1/500th of basal dose) and obtained similar growth promoting effect. Likewise, Ahmadi et al. [65] observed increased body weight gain, feed intake, and feed efficiency at 60 and 90 mg/kg diet of ZnO NP, but exhibited a lower performance in chickens at a dose of 120 mg/kg diet, whereas Fathi [66] reported lower feed efficiency in birds by supplementing nano-ZnO at 40 mg/kg. In contrast, feed intake, body weight gain, feed efficiency and, carcass traits were not affected due to supplementation of nano-ZnO at 25 and 50 mg/kg as well as ZnO at 100 mg/kg [50]. Nano-Zn at 80 mg/kg increased Zn, Ca and P levels, bone dimensions, weight, total ash along with higher liver and muscle Zn concentration [74]. Significantly lower fat and cholesterol content and better antioxidant status was obtained by supplementation of nano-Zn than inorganic Zn at 80 mg/kg [74]. Nano-ZnO increased mRNA expressions of insulin like growth factor-1 and growth hormone genes in broiler chickens compared to the inorganic or organic Zn [75].

Nano-Cr increased protein contents in thigh and breast muscles and lowered fat and cholesterol concentrations in thigh muscles. Chromium NP at 0.5 mg/kg diet improved breast and thigh muscle protein content, average daily gain and feed efficiency and lowered cholesterol and fat in thigh muscles of the broilers [76]. However, supplemental nano-Cr picolinate at 0.5 and 3 mg/kg [58] or 0.4 mg/kg [59] of Cr did not affect body weight, feed intake, feed efficiency, and egg production of layer birds. Supplementation of nano-Cr at 0.8 mg/kg diet was effective in reducing the negative effects of induced stress on meat quality of quail broilers, as evident from reduced malondialdehyde concentration in the thigh muscle and unaltered hematological parameters [77]. CuO NP was more efficient in increasing growth performance and immunity compared with the conventional sources of Cu [78]. As compared to the inorganic salts, supplementation of nano-Se improved the body weight gain and lowered the feed to gain ratio at doses up to 0.30 mg/kg diet, beyond which no beneficial effect was recorded [52, 54]. Se-yeast and nano-Se resulted in better growth performance than sodium selenite at 0.2 mg/kg Se [79]. Nano-Se supplementation reduced drip loss percentage without altering weight gain, feed intake, and feed conversion, meat color or immune organ index (thymus, bursa, and spleen) in broilers [57]. However, Se supplementation irrespective of its source (sodium selenite or nano-Se) increased daily weight gain and feed efficiency [67], though nano-Se proved to be more efficient. Improved weight gain and feed conversion due to supplementation of Fe NP (7 mg/kg) was possibly due to increased arginine in liver [80]. Dietary Fe sulfate NP resulted in highest jejunal villi width and surface area in broiler chickens at 21 and 42 days of age [81]. Overall, above studies that nano-minerals at reduced dose rates have potential to improve growth performance and the quality of meat compared with the conventional mineral sources.

### 9. Impact of nanominerals on layers

Eggs and meat are the primary products of poultry industry which are widely accepted, consumed and provides the quality nutritional security to the human race. The huge demand of poultry products intensifies the production system and improving the productivity becomes the major focus of research. The composition and nutrient density of poultry diets affect composition and nutritional quality of eggs and their products [82]. Considering the layer birds, precise mineral nutrition is very important to maintain the egg production and egg production is very susceptible to minute deficiency of many minerals. Several studies have been conducted to validate the effects of nanominerals in layers and their effects on egg productions are documented.

Calcium is required for egg shell formation; hence its requirement in layer diets is very high to sustain egg production. On adding 1 g/L of Ca carbonate NP in water, Wang et al. [83] could find stronger eggshell strength and better freshness indexed eggs as compared to the control. However, Ganjigohari et al. [84] reported a drop in egg production percentage, egg mass and low blood Ca level by reducing 4.03% of Ca carbonate by 0.126% Ca carbonate NP in laying hens as compared to birds supplemented with 4.03% of Ca carbonate replaced by 2.02%, 1.01%, 0.25% Ca carbonate NP, which would be due to too much reduction in the Ca level in the bird's diet. Similarly, Zn supplementation is obligatory to improve egg production and quality [1]. Being a part of enzyme carbonic anhydrase, Zn is required during egg shell formation and thus deficiency of Zn results in poor eggshell quality. Zn also interrelate Ca crystals during eggshell synthesis [85] which indirectly affects formation of shell membrane and eggshell. Abedini et al. [85] reported an improvement in feed intake, egg mass, egg Haugh unit, eggshell thickness and strength, and tibia ash content and strength at 40 and 80 mg Zn/kg diet as ZnO NP in the laying hens. Tsai et al. [86] observed a hike in Zn retention, thickness of the eggshell, concentration of growth hormone in the blood serum and carbonic anhydrase in nano-Zn supplemented groups as compared to control, without affecting the immunity and other nutrient retention in birds. However, no effect of different dietary Zn sources such as inorganic, organic or nano-Zn on egg quality parameters namely, egg mass, eggshell weight, and eggshell breaking strength [87, 88]. An increase in Zn content in egg yolk due to supplementation of nano-Zn may be proved advantageous to produce designer egg and aid in better keeping quality [82]. Olgun and Yildiz [89] observed highest egg weight and the lowest eggshell thickness by supplementation of nano Zn at 50, 75 and 100 mg per kg diet as compared to Zn-sulphate and Zn-oxide and Zn-glycine supplemented birds and thus, suggested nano-Zn supplementation negatively affects the eggshell thickness and bone mechanical properties. However other studies reported positive responses by supplementation on nano-Zn [82, 85]. The malondialdehyde content in egg was reduced in the groups supplemented with Zn-oxide NP at 80 mg Zn/kg diet [87] and 40 mg/kg of Zn-oxide NP [66], respectively. Dietary Se has a significant role in egg production and immunity in poultry and reports suggests better responses of nano-Se as compared to its conventional counterparts. Radwan et al. [56] reported that use of nano-Se improved Se content in eggs, egg production and feed conversion ratio without affecting egg weight, feed intake in birds. Qu et al. [90] supplemented Se-NP at 0.5 mg/kg diet in laying hens and reported improved rate of egg production, glutathione peroxidase (GPx) activity, total antioxidant status, along with decreased soft-shelled or cracked egg rate. Meng et al. [53] observed an increase in egg Se concentration by supplementation of Nano-Se at 0.3 mg/kg as compared to sodium selenite, Se-yeast. However, they observed that Se-yeast and nano-Se were better retained in the body than sodium selenite. It is observed that injection of nano-Se and nano-ZnO lessened the negative effects of heat stress by increasing antioxidant activity and reducing oxidative stress [91]. Shokraneh et al. [91] observed increased activity of GPx and superoxide dismutase (SOD) and total protein and decreased the levels of corticosterone, cortisol, T4 and T3 in the eggs being injected with NaCl solution containing 40 µg nano-Se at high eggshell temperature. In ovo injection of Cu-NP on 1st and 10th day of incubation showed decreased oxygen consumption, lower heat production, higher residual yolk sac weight compared with the control group, signifying reduced lipid oxidation in Cu-NP injected group [92]. Supplementation of nano-Cu in poultry has shown varied effects depending on dose administered. For an instance, in ovo supplementation of nano-Cu and Cu-sulphate NP at 50 mg hiked the red blood cells and white blood cells in poultry [93], while at a dose of 0.3 mL containing 50 mg/L nano-Cu improved the expression of pro-angiogenic

and pro-proliferative genes [94], and also improved the bone characteristics at 42 days birds which signified the possible effect of nano-Cu in bone development and maintenance [95]. The better performances in poultry by in ovo injection of nano-Cu was attributed to improved metabolic rate during embryogenesis resulting in amplified performance of broiler chickens after hatching [96]. Supplemental nano-Cr-picolinate at 0.5 and 3 mg/kg of Cr improved egg quality, retention of Cr and Zn, whereas, it decreased shell ratio in the 60th day eggs [58]. Nano-Cr supplementation at 0.4 mg/kg of feed also improved egg quality parameters including Cr and Zn content in eggshell, Ca in the liver and eggshell, and Zn in egg yolk [59]. Another study specified that eggs of birds supplemented with nano-Cr at 0.2 and 0.4 mg/kg feed exhibited higher shell strength than eggs of hens from the group control and receiving Cr in an inorganic form [97]. In ovo supplementation of nano-Fe though improved body weight gain to egg ratio and feed conversions ratio at 7 days of age in broiler chicks, but no such improvement was observed later and there was no significant effect on hematological parameters [98]. L-cysteine-coated Fe-oxide NP at 6 or 60 mg/kg diet improved availability and utilization of Fe as evident from increased percentage of egg production and egg weight in quails [99]. Taken together, nano-minerals can improve egg production and s eggs quality traits at decreased rate of application.

# 10. Impact of nanominerals on anti-oxidative activity

Trace minerals particularly Zn, Se, Cu, and Mn play a major role in anti-oxidant system, either being a component of the antioxidant metallo-enzymes or indirectly by regulating their activities. These enzymes act an indicator of the wellbeing of the animals and they increase or decrease depending on the mineral level in the animal or birds. Hence, better bioavailability of a mineral source can be determined by monitoring these enzyme levels which are mineral specific. Different mineral NP have been shown to improve antioxidant status in birds compared to their conventional forms (**Figure 3**). Supplementation of ZnO-NP at 40 and 80 mg/kg in the diet of broiler chickens augmented their antioxidant status as evidenced from increased activity of SOD and catalase, and decreased concentration of malondialdehyde [100]. Zhao et al. [17] observed higher total antioxidant activity in serum and liver tissue, serum catalase activity and reduced serum and liver malondialdehyde concentration in the 20 mg/kg nano-ZnO group of broiler chickens

Nano-selenium Improves Se retention, growth, immunity, egg production, antioxidant enzymes, ccarcass characteristics, higher safety	Nano-calcium phosphorus Improves growth, feed efficiency, immunity, egg production, antioxidant enzymes	Nano-zinc Improves Zn retention, growth, feed efficiency, egg production, antioxidant enzymes, carcass characteristics, antimicrobial
Mano-chromium •Improves Cr Retention, •Growth and meat parameters •Egg quality and production parameters •Feed efficiency		agent, less toxic than micro particles <u>Nano-silver</u> Improves immunity, reduces chances of infection and loads of pathogenic microbes, increases beneficial microbes in gut
<u>Nano-iron</u> Improves Fe retention, growth, immunity, hatching, fertility,		Nano-copper Improves Cu retention, growth, Immunity, antioxidant enzymes

### Figure 3.

Beneficial effect of different nanominerals in poultry production.

compared to the control (60 mg/kg ZnO) chickens. Zhao et al. [17] also reported higher activity of serum and liver Cu-Zn-related SOD and serum catalase in the diets containing 60 and 100 mg Zn/kg from ZnO NP. Bami et al. [50] reported that ZnO NP at 25 and 50 mg/kg as well as conventional ZnO at 100 mg/kg did not affect feed intake, body weight gain, feed efficiency and, and carcass traits, but higher dose of ZnO NP lowered malondialdehyde content and cooking loss in meat compared with 100 mg/kg of ZnO. Lina et al. [101] also observed increased GPx activity, total antioxidant activity in serum and reduced serum malondialdehyde content in Arbor Acres broiler chickens at 40 mg/kg of Zn supplementation from nano-ZnO. At higher levels (80 mg/kg) of Zn supplementation through either green nano-Zn or commercial nano-Zn in broiler birds showed significantly higher serum SOD, GPx, and catalase levels than their 40 and 60 mg/kg diet and inorganic Zn at 40, 60 or 80 mg/kg diet [74]. Ahmadi et al. [65] observed significantly increased SOD activity at 60–90 mg Zn/kg diet from ZnO NP in broiler chicken ration compared to 30 mg/kg diet. However, they observed inhibitory action of nano-Zn at further increased level (120 mg/kg diet) apparent from decreased SOD activity. Supplementation of 0.19 mg Se/kg diet from Se-NP increased serum SOD and GPx activity and decreased malondialdehyde concentration compared to 0.15 mg Se/ kg diet from coarse Se in broiler birds [102]. Nano-Se at 0.3 mg/kg diet expressed improved GPx activity, free radical inhibition, immunoglobulin M concentration and decreased glutathione and malondialdehyde content in serum, improved GPx activity and free radical inhibition in liver and GPx activity in muscle [57]. Elkloub et al. [70] observed better total serum antioxidant activity in Ag-NP supplemented groups (2, 4, 6, 8, 10 mg/kg) and best in 4 mg/kg diet in Ag-NP supplemented group than un-supplemented group. Jankowski et al. [103] observed no adverse effect on antioxidant defense on reducing Mn concentration from 100 to 10 mg/kg diet in the form of NP-Mn2O3 whereas a 50% reduction in Mn level in the form of MnO enhanced lipid oxidation processes in turkeys.

# 11. Effect of nanominerals on health and immunity

Trace essential minerals also act as immune stimulants in birds. Hence balanced mineral mixture is given much priority to maximize the animal or bird productivity and minimize the stress (biotic as well as abiotic) in animals and birds. Minerals particularly Zn, Cu, Se and Mn are studied on their immune-stimulant effects profusely, which may require at higher concentrations for better immunity compared with the optimum production levels [1, 2].

Different nanominerals have showed to exhibit better immune responses (**Figure 3**). Hafez et al. [100] observed enhanced cellular immunity evidenced from increases in serum IgY concentration, total lymphocyte count, macrophages, phagocytic activity and phagocytic index in ZnO-NP fed groups compared to ZnO supplemented group. Nano-Zn supplementation at 0.06 mg/kg in the basal diet improved immune status of broiler equivalent to that of 15 mg/kg diet of organic Zn supplementation in term of increased weight of lymphoid organs and improved humoral immunity [47]. Supplementation of ZnO-NP in dry broiler ration improved carcasses yield and relative weight of lymphoid and digestive organs compared to wet diet during the starter period [104, 105]. Retention of Se in liver and muscle increased in a dose dependent manner with dietary intake of nano-Se (0.3, 05, 1 and 2 mg/kg diet), but did not affect growth performance whereas improved meat quality, immune function, and oxidation resistance were observed for nano-Se level ranging from 0.3 to 1 mg/kg diet [57]. Addition of Cu-NP in drinking water (10 mg/l) improved immunity, and productivity more efficiently compared to coarse CuSO4 [106]. A study involving Cu-NP, agglomerates of Cu-NP and Cu microparticles on the metabolism in broiler chickens after a single intramuscular injection revealed that all these forms had growth stimulating effect along with increased red cell level, hemoglobin, Cu and protein in blood serum, where effects were expressed quickly by Cu-NP [107].

# 12. Nanominerals as antimicrobial feed additives

Pathogenic microbial load in the gut of poultry is detrimental as they reduce growth rate, feed efficiency and mortality, and are some of these contaminants may survive during food processing and storage. The in-feed antibiotics used for preventing the pathogens as well as growth promoters has been great concern due to possible emergence of drug resistance in microbes as well as appearance of the drug residue in poultry products and subsequently affecting consumer's health [108]. There is an optimism of using nano-minerals as antibiotic alternatives due to their antimicrobial properties [109]. NP use has been established in therapeutics, drug delivery and diagnostics [110].

Many research carried has explored the antimicrobial action of metal oxide-NP [109]. Nano-Ag supplementation at 4 mg/kg diet in broiler chickens reduced serum cholesterol, aspartate aminotransferase levels and reduced caecal *Escherichia coli*, but had no significant effect on Lactobacillus count [70]. Nano-Ag in water (25 mg/ kg) increased the population of lactic acid bacteria without any adverse effect on enterocytes of duodenal villi [111]. Nano-Ag lowered the number of *Escherichia coli*, *Streptococcus*, *Salmonella*, and total mesophilic bacteria in the litter [112]. ZnO-NP was found be effective against both gram positive and gram-negative bacteria [109, 113] as well as spores that are resistant to high temperature and pressure [29]. ZnO-NP are also effective in inhibiting the growth of fungi (*Aspergillus flavus*, A. ochraceus and A. niger) and their mycotoxins production [114]. Some possible mechanisms of bactericidal action of metal oxide NP was generation of reactive oxygen species inside the bacterial cells (e.g., hydroxyl, hydroperoxide, and superoxide radicals) that may damage lipid membranes of cells and organelles of bacteria [109, 115] or they alter permeability of bacteria after entering their plasma membrane resulting in cell death [116]. They may damage bacterial cell after penetration by interacting with sulfur and phosphorus containing important compounds like DNA [113]. As per Rajendran et al. [117], ZnO NP inactivates the proteins that are responsible for transport of nutrients, thus decreasing the membrane permeability and eventually causing the cellular death. Another explanation about antimicrobial action of metal oxide NP is that microorganisms carrying a negative charge are electromagnetically attracted towards metal oxides carrying a positive charge, subsequently leading to oxidization and death of microbe [113]. The antibacterial activity depends on the size, with better result obtained with smaller size [118]. But, Arabi et al. [113] observed the significance of surface area and concentration of NP, whereas crystalline structure and shape of NP have little significance.

### 13. Environment implication of nanomineral supplementation

In high intensified production system, trace minerals are added to poultry diets in high amounts exceeding the birds' requirements, with a large safety margin, creating environmental issues particularly in areas of intensive poultry production [8]. A study reported that by using poultry manure, Zn content in soil was found in excess by 660% in comparison to plant Zn requirements, predisposing to

phytotoxicity [119]. Among minerals, Fe, Cu and Mn are always found in excess than the amount required by the plants [8].

Poor retention efficiency on inorganic mineral sources leads to excretion of unabsorbed minerals to the environment, which may be a potential environment pollutant especially in the area of intensive poultry farming. Phosphorus excretion in poultry excreta from large scale poultry farming has been a matter of concern from environment pollution point of view. Predominantly, inorganic P sources are used in poultry ration for optimum growth, egg production which results in excretion of huge amount of P to the environment [1]. Improved absorption of minerals through NP may reduce excretion of unutilized minerals and could minimize the environmental pollution. Cufadar et al. [88] reported that the Zn content of the feces was less in poultry when they are supplemented with nano-Zn as compared to organic and inorganic Zn. Furthermore, Hassan et al. [69] found that DCP NP supplementation reduced excretion of Ca and P by 50.7 and 46.2%, respectively. Dietary Cu is absorbed in a range between 10 to 30% in the small intestine of the animal creating environmental issues [3, 92], but supplementation of nano-Cu has been proved effective in prevention of environmental leaching of unabsorbed Cu by virtue of its efficient intestinal absorption and also functions at the molecular level [120, 121]. Considering Zn, predominantly ZnO or ZnSO4 are used in commercial poultry feeds and among these, ZnO is used in 80–90% cases having less bioavailability [122]. Reports suggests nano-Zn are better absorbed in different animals [40, 41] and birds [47, 48], thus reducing the amount of Zn excreted to the environment, and environment pollution. Reports suggest that nano-Cr has better bioavailability than organic and inorganic Cr supplements [123]. Therefore, nanominerals offer opportunities to reducing environmental pollution of minerals without compromising the production, health and quality of products.

# 14. Special aspects of nanominerals

Trace minerals such as Se is essential in minute quantities in poultry diet to reduce stress, improve immunity and overall health, but safe limit of inorganic Se is very narrow. Hu et al. [51] observed a wider range between the optimal and toxic dietary levels of nano-Se compared to inorganic sodium selenite in broiler chickens. But contradictory reports exist regarding occurrence of severe pathological changes in liver due to increase in nano-Se concentration from 0.15 to 0.3 mg/ kg diet [124]. One of the possible mechanisms of nano-Se action in poultry could be conversion of nano-Se into selenite, H2Se or Se-phosphate followed by synthesis of selenoproteins by gut microbiota, which was also reported by Surai et al. [125] in *Veiollonella*. Reports suggest lower toxicity of Se NP than selenomethionine [126]. Also, Gangadoo et al. [127] reported that nano Se did not cause any damage to epithelial cells in the digestive system and neuronal bodies in brain tissue signifying its lesser toxicity in animal models. Nano-Ag in higher concentration (8 and 12 mg/ kg diet) had detrimental effect on organs such as liver [128]. Ag-NP supplementation in drinking water (15 mg/L) of broilers had no significant growth promoting or coccidiostat action [129]. At higher concentration (50 mg/L) in drinking water, no effect on the intestinal colonization of C. jejuni was found; however, reduced body weight gain was observed in broiler chickens [130]. Dietary ZnO NP at 40 and 60 mg/kg alleviated the negative results of heat stress evident from lowering of serum corticosterone level [131]. Saki and Abbasinezhad [132] reported improved embryonic growth and development in broiler on supplementation of 25 mg/kg diet of nano-Fe and 100 mg/kg diet of Fe-nano-alimet chelate. But toxicity of nano-FeO was reported in chick embryo possibly due to its interaction with egg albumen.

FeO NP caused 100% mortality at 200 mg/L and decreased body weights and crown-rump lengths of embryo at 50 and 100 mg/mL and 50–60% degeneration of neurons in brain at 10–100 mg/L dose range [133]. It seems few minerals in their nanoforms are more toxic than their coarser forms. Therefore, safety and toxicity levels of different nanominerals should be widely evaluated before recommending them at an optimum dose for their use in poultry.

# 15. Other nanoparticles in poultry nutrition

Many other nanomaterials have been used in poultry nutrition. For instance, turmeric extract enclosed in a nanocapsule improved meat quality traits without affecting performance as a feed additive for regular broiler feed and 0.2% turmeric nanocapsule was the optimum level to obtain the best feed efficiency, whereas 0.4% of the nanocapsule decreased liver cholesterol and subcutaneous fat, but concentration at >0.4% reduced growth in birds [134]. Clay minerals of nano-suspensions added at 1 to 2% of the suspension in drinking water in broiler chickens were found to improve feed conversion ratio, body weight gain and antibody titer against Newcastle disease, infectious bronchitis and bursal disease [135]. There is huge potential of functionalizing many nanoparticles for application in poultry nutrition and feeding [32].

# 16. Conclusions

Minerals are obligatory for maintaining the higher productivity of poultry and a better bioavailable source at lower cost is the prime priority. In this context, nanominerals have produced encouraging responses in most of the studies. Though inconsistent, nano form of Zn, Se, Cu, Ca, and P mostly produced improved responses in poultry reflected by their performances such as body weight gain, feed efficiency, immune responses, egg production, egg quality traits, bone quality parameters, retention of minerals, and enzyme level. The inconsistent performance may be attributed to the level of minerals present in feeds itself and also the varied doses used depending on the hypothesis of the researchers. Many cases have given similar responses even by reducing the dose to half of the conventional inorganic doses, which is suggestive of better bioavailability. Apart from the biological effects in birds, the nanominerals are found to reduce the environmental excretion of the minerals by virtue of its better bioavailability and also reducing the dose of supplementation as well. Considering all the aspects, this can be suggested that use of nanominerals in poultry ration can be considered as an environmental protective strategy to augment poultry productivity. However, further studies with more replicates should be advocated along with long term exposure to validate and unveil more aspects of nanominerals.

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