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Review article

Nano zinc, an alternative to conventional zinc as animal feed supplement: A review

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ABSTRACT

The uniqueness of Zn is that, it is the second most abundant trace element in the animal body but can't be stored in the body, thus regular dietary intake is required. Zinc oxide (ZnO) nanoparticles (NP) particles are being extensively used in paints, skin lotions pigments, food, electronics appliances, biological and pharmaceutical applications and many more. Zinc oxide nanoparticles are the specially prepared mineral salt having particle size of 1 to 100 nm. It promotes growth can act as antibacterial agent, modulates the immunity and reproduction of the animals. Both in lower and higher doses of specifications it has exhibited a variety of effects on animal performances. Apart from being highly bioavailable, reports have already pointed out the growth promoting, antibacterial, immuno-modulatory and many more effects of nano zinc (nZn). These can be used at lower doses and can provide better result than the conventional Zn sources and indirectly prevents environmental contamination also. The toxicological studies provide mixed results in animal models. Studies been undertaken in diversified animal species and encouraging effects have been reported with nZn supplementation. However, there is a need to optimize the dose and duration of ZnO NP supplementation for human and livestock, depending on its biological effects. Actual bioavailability of ZnO NP in livestock is still to be worked out. In this review we have attempted to summarize, conclude the beneficial effects of nZnO and its possible usage as mineral supplement to different categories of human and livestock.

1. Introduction

Among metal nanoparticles (NP) annually produced, by volume, nano zinc oxide (nZnO) is the third highest globally produced nano metal after nano SiO_2 and nano TiO_2 (Piccinno et al., 2012). The sudden rise in the demand in zinc oxide nanoparticles (ZnO NP) is mostly attributed to its better antibacterial properties than the conventional ZnO (Padmavathy and Vijayaraghavan, 2008). Zinc oxide nanoparticles are being used in the food industry as additives and during packaging due to their antimicrobial properties (Gerloff et al., 2009; Jin et al., 2009). Studies have already proved the dose dependant effect of ZnO NP on growth performance in livestock and poultry (Hongfu, 2008; Yang and Sun, 2006; Lina et al., 2009a, b; Mishra et al., 2014; Sahoo et al., 2014a,b) and also as antimicrobial and immune-modulatory agent by reducing the diarrhoea rate in piglets (Hongfu, 2008).

Nanotechnology has revolutionized the commercial application of nano sized minerals in the fields of medicine, engineering, information, environmental technology pigments, food, electronics appliances, biological and pharmaceutical applications and many more. This is also been used as a recent tool in the fields of biology (molecular and cellular), biotechnology, mineral nutrition, physiology, reproduction, pharmacology etc. in both animal and human models. Furthermore, it can be used for pathogen detection. Thus,

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there are diversified areas and use of nanotechnology including the science and engineering of agriculture, animal and food systems. Economies of many countries depend on agriculture and nanotechnology is important for future animal husbandry and feeding (Sri Sindhura et al., 2014). Nanotechnology is concerned with materials whose structures exhibit significantly novel and improved physical, chemical, and biological properties, phenomena, and functionality due to their nano scaled size (Wang, 2000). It can be defined as a research and development aimed at understanding and working with seeing, measuring and manipulating matter at the atomic, molecular and supramolecular levels (NSTC, 2004). These NP refer to a particle size of roughly 1 to 100 nm (Feng et al., 2009). At this scale the physical, chemical and biological properties of material differ fundamentally and often unexpectedly. These nanomineral particles are having higher potential than their conventional sources and thus reduce the quantity required (Sri Sindhura et al., 2014). The added advantage is ZnO NP can efficiently be synthesized by using any of physical, chemical or biological methods (Swain et al., 2015) which are cheap and easy. The aim of the present review is to present current scenario with regard to significance of zinc for livestock, bio-availability of zinc, effectiveness of NP on different livestock and lastly but not the least toxicity of NP need to be ascertained before it is recommended for livestock.

1.1. Significance of zinc

Zinc (Zn) is the second most abundant trace element in the animal body. It can't be stored in the body (Zalewski et al., 2005) and requires regular dietary intake to meet the physiological needs. The importance of Zn on human and animal health has been documented many years ago (Table 1). As a component of numerous enzymes and hormones, Zn is necessary for the proper physiological functioning (Prasad, 1991). These includes alcohol dehydrogenase, alkaline phosphatase (ALP), aldolase, lactate dehydrogenase (LDH), RNA and DNA polymerases, reverse transcriptase, carboxypeptidase A, B, G and superoxide dismutase (SOD). Zn is essential for body's proper physiological functions like, normal growth (Case and Carlson, 2002), reproduction (Uchida et al., 2001), DNA synthesis, cell division and gene expression (Prasad, 1991), photochemical processes of vision (Suchý et al., 1998), wound healing (Zhao et al., 2014), ossification (Roughead and Kunkel, 1991), augmenting the immune system of the body (Zhao et al., 2014; Parashuramulu et al., 2015) through energy production, protein synthesis, protection of membranes from bacterial endotoxins and lymphocyte replication and antibody production (Nockels, 1994). Zn is a component of the free radicals scavengers which are produced during different physiological processes (Zhao et al., 2014), and is also required for the normal condition of epidermis, epithelium, skin and hooves (Kruczynska, 2004). It has been observed that rats and humans are susceptible to even marginal Zn deficiency which reduces immune responses (Fraker et al., 1984) but in ruminants (Droke and Spears, 1993), marginal Zn deficiency does not impair cell-mediated or humoral immune responses (Spears, 2000). There is an increase in the immunoglobulin level in colostrum as well as in blood serum by supplementing organic Zn (Kinal et al., 2005). Zn plays an important role in the formation of insulin (Kruczynska, 2004). Role of Zn on livestock reproduction came into picture when Mussill (1941) reported that, sterility in heifers was attributed to insufficient Zn. It is having a synergistic effect on the reproductive performances of the animals. Thus Zn is routinely supplemented in human and livestock foods and feeds for normal physiological functions as well as to meet the daily requirement.

Table 1
A brief view on the essentiality of zinc (Zn).

Species	Systems under investigation	Effect and conclusions	References
Rabbit	Reproduction	Increased semen volume, total live sperm concentration, per cent sperm motility, conception rate in heat stressed rabbits.	El-Masry et al. (1994)
Human, lab animals	Immunity	Reduced immune responses and disease resistance in Zn deficient subjects.	Chesters (1997)
Goats	Immunity	Enhanced resistance to udder stress in dairy goats to Zn supplementation.	Salama et al. (2003)
Mice	Immunity	Alcoholism reduces Zn transporter gene expression thus reduces immunity compared with normal subjects.	Sun et al. (2014)
Mice	Vision	Accumulation of inclusion bodies in the retinal pigment epithelium, cause its alterations due to Zn deficient diets.	Leure-dupree and Mc Clain (1982)
Mice	Epithelial cells	Enhances proliferation of the cells and did not injure the cells at lower concentrations; impacts on epithelial cell integrity of the animals.	Feng et al. (2009)
In vitro and *in vivo*	Antioxidant	Exhibits antioxidant-like effects *in vitro*. At pharmacological doses *in vivo*, Zn has a protective effect against pro-oxidants and dietary Zn deficiency predisposes to oxidative damage in cells by protection of sulfhydryl groups and inhibits production of reactive oxygen species (ROS) by transition metals.	Bray and Bettger (1990)
Ruminants	Reproduction	Higher incidence of abortions and stillbirths in Zn deficient ewes	Campbell and Mills (1979); Najafzadeh et al. (2013)
Poultry (Broilers)	Growth, carcass traits and meat quality	Increased ADFI, ADG, DM and intramuscular fat contents of the breast muscle, percentage of eviscerated yield, redness value in breast muscle and pH values in thigh muscle and decreased shear force in thigh muscle, drip loss in breast and thigh muscle.	Liu et al. (2011)

1.2. Bio-availability of Zn

Absorption of Zn in the body is very less and differs with the age of the animal and the sites in the gastrointestinal tract. The net absorption of Zn administered daily was different in mature cows (12%), 5 to 12 months calves (20%) and also in week-old calves (55%). In the animal's body, Zn is mostly absorbed from the abomasum and lower small intestine (Miller and Cragle, 1965). Absorption below the cecum is insignificant and secretion of endogenous Zn occurred from the upper part of the small intestine (Miller and Cragle, 1965). Zn can be incorporated in the diet as inorganic salts like ZnO and Zn Sulphate ($ZnSO_4$) and as organic chelates such as Zn propionate and Zn acetate. Even though, the bioavailability of Zn in organic sources is higher than that of inorganic Zn salts, the use of organic Zn chelates in animal diets is limited due to its higher cost (Zhao et al., 2014). Higher levels of Zn excreted from the supplemented animals have raised concerns pertaining to environmental pollution (Feng et al., 2009). Thus, this problem opens a window for better bio-available Zn sources and if possible, to reduce the supplemental dose of Zn to the animal food. Among all the probable approaches, use of nanotechnology to produce nano sized Zn called as nano Zn (nZn) is a potential alternative to both organic and inorganic Zn sources. The use of nZn has shown to produce better results as compared with conventional Zn sources and also micro Zn and is also less toxic (Wang et al., 2006; Sahoo et al., 2014b).

1.3. Properties of nano minerals

Nano minerals, dimensions below 100 nm is called nanomaterials, are stable under high temperature and pressure (Stoimenov et al., 2002). By virtue of their small size, it is easier to be taken up by the gastrointestinal tract, so are more effective than the larger size ZnO at lower doses (Feng et al., 2009). In the animal body, nano minerals interact more effectively with organic and inorganic substances due to their larger surface area (Zaboli et al., 2013). Zinc oxide nanoparticles also have minimal adverse effect on human cells (Reddy et al., 2007). Nano minerals have the capability to cross the small intestine and further distribute into the blood, brain, lung, heart, kidney, spleen, liver, intestine and stomach (Hillyer and Albrecht, 2001). The functional activities such as chemical, catalytic or biological effects of NP are heavily influenced by the particle size of the nanometals (Rosi and Mirkin, 2005). Zinc oxide nanoparticles were mainly found to be retained in the liver after 14 day sub-acute exposure (Sharma et al., 2012) and oral administration through gastrointestinal tract.

2. Effect of the ZnO NP supplementation on biological systems

Just like the conventional sources, nZn also plays very significant role in animals (Table 2). Though there is scanty literature on this important subject, we have made an attempt to synthesize the outcomes and the conclusions of the various studies done on nZn worldwide.

2.1. Growth

Zinc oxide nanoparticles has been reported to enhance growth performance, improve feed utility and provide economic benefits in weaning piglets and poultry (Yang and Sun, 2006; Mishra et al., 2014). Encouraging results in average daily gain was obtained by feeding basal diets supplemented with 200, 400, 600 mg/kg nZnO or 3,000 mg/kg ZnO (Hongfu, 2008). Zinc oxide nanoparticles has been found to improve the production performance and dressing performance of broilers on 42 days of feeding at the level of 40 mg/kg in the diet (Lina et al., 2009a). Mishra et al. (2014) observed a significant improvement in growth rate in layer chicks than inorganic Zn even at 1/500 of nano Zn level of basal dose and also observed an increase in levels of serum glucose and ALP and a decrease in alanine aminotransferase (ALT) at this level of nano Zn supplementation. In ruminants, large doses of Zn cannot act as growth promoters, however, doses up to 3,000 mg/kg feed have been proved to be growth promoting in pigs (Hongfu, 2008). Hongfu (2008) studies the effect of Nano-ZnO on weanling piglets growth performance and diarrhoea rate and reduced doses of nZnO (200, 400, 600 mg/kg) as a substitute for high doses of inorganic ZnO (3,000 mg/kg) and reported that The basal diets supplemented with 400 mg/kg ZnO NP reduced the diarrhoea rate by 49.1% which showed a nonsignificant differences with the piglets supplemented with 3,000 mg/kg ZnO.

2.2. Milk production

Nano Zn has been reported to reduce the somatic cell counts in cows with subclinical mastitis and improve milk production compared with other conventional ZnO sources. Thus, nano Zn may

Table 2
Effect of nano zinc on animal performance.

Serial no.	Species	Effects	Remarks	References
1	*In vitro* supplementation of 100 and 200 mg/kg of ZnO NP at the 6th and 12th h of incubation	Fermentation	Improved concentration of volatile fatty acid and microbial crude protein production and fermentation of organic matters. Concentration of ammonia nitrogen and the ratio of acetate to propionate are adversely affected.	Chen et al. (2011)
2	Pig	Immunity	Diarrhoea incidence reduced.	Hongfu (2008); Yang and Sun (2006)
3	Cattle (*Holstein Fresien*)	Milk production	Reduce somatic cell count in subclinical mastitis. Increase in milk production.	Rajendran et al. (2013)
4	Poultry (Broilers)	Growth; Feed consumption	Improves in growth performance, FCR and dressing performance; decrease in the cost of production.	Lina et al. (2009a)
5	Sheep	Reproduction	High incidence of abortions and stillbirths in the ewes in ZnO NP deficient diets.	Campbell and Mills (1979); Najafzadeh et al. (2013)
6	Poultry	Growth	Improves growth performance and FCR.	Hongfu (2008); Yang and Sun (2006)

ZnO NP = zinc oxide nanoparticles.

be used both as preventive and curative agent to control subclinical mastitis in cows (Rajendran et al., 2013).

2.3. Rumen fermentation

Chen et al. (2011) studied the impact of nZnO (0, 50, 100, 200, 400 mg/kg of DM) supplementation of rumen fermentation pattern. Supplementation of ZnO NP, *in vitro* has been reported to improve the growth of ruminal microorganisms, increase the ruminal microbial protein synthesis and raise the energy utilization efficiency in early phase (6 to 12 h) of incubation (Chen et al., 2011). There is an increase in the concentration of volatile fatty acid, microbial crude protein production and the fermentation of organic matter while the concentration of ammonia nitrogen and the ratio of acetate to propionate are adversely affected by the supplementation of 100 and 200 mg/kg of ZnO NP at the 6th and 12th h of incubation *in vitro* (Chen et al., 2011).

2.4. Immunity

In human as well as in lab animals, Zn deficiency reduces immune responses and disease resistance (Chesters, 1997). But the role of Zn as an antioxidant in the central nervous system, particularly the brain is gaining attention in recent times. Zinc is essential to the structure and function of myriad proteins which are classified as regulatory, structural and enzymatic. In the central nervous system, zinc has an additional role as a neurosecretory product or cofactor. In this role, zinc is highly concentrated in the synaptic vesicles of a specific contingent of neurons, called "zinc-containing" neurons, which is a subset of glutamatergic neurons which are exclusively present in forebrain (Frederickson et al., 2000).

Significant improvements were observed in the health status (low blood cholesterol level and high ALT) and immunity of the birds by supplementing nZn to broiler diets at 0.06 mg/kg compared with the conventional dose of 15 mg/kg of organic and inorganic Zn with the basal diet (Sahoo et al., 2014a,b). Compared with other soft tissues, the human brain contains significant amounts of Zn. By supplementing basal diets with 400 mg/kg ZnO NP, diarrhoea rate was reduced up to 49.1% compared with 21.6% upon supplementation of 3,000 mg/kg ZnO (Hongfu, 2008).

2.5. Reproduction

Nano sensors are available to study the causes of abortion. Nano antioxidant is one of the area to be explored to prevent retain placenta and other reproductive problems after calving and for improving infertility problems. Zinc has antioxidative properties and plays an important role in scavenging reactive oxygen species. Absence of Zn may cause increased oxidative damage exists that may contribute to poor sperm quality (Colagar et al., 2009). Zn controls the energy utilization through ATP system involved in contraction and regulation of phospholipid energy reserves, thus influences motility of spermatozoa (Hidiroglou and Knipfel, 1984). Zn controls the motility of goat sperms by influencing development of flagella of sperm tail (Saleh et al., 1992). Roy et al. (2013) reviewed that Zn is important for sperm motility and viability. In sperm middle piece, Zn is involved in catabolism of lipid, and thus is the source of energy for motility of spermatozoa. Ahmed et al. (1997) reported in buffaloes that, high concentration of Zn in the spermatozoa is essential for their viability and fertility. Poor Zn nutrition may be an important risk factor for low quality of sperm and idiopathic male infertility (Colagar et al., 2009). New application in animal production system is nanotube implanted under the skin to provide real time measurement of level of estradiol in the blood. Zn deficient diets are a cause of high incidence of abortions and stillbirths (Campbell and Mills, 1979; Najafzadeh et al., 2013). Supplementation in the form of nZn to animals can possibly eliminate these reproductive disturbances and thus may improve the economics of farming. So studies must be done in this aspect to explore the possibilities of nZn in augmenting animal reproductive performances.

2.6. Antibacterial activity of nZn

Many researchers have pointed out the antimicrobial action of metal oxide NP (Table 3). Antibacterial activity means the reagent that locally kills bacteria or slows down their growth, without being toxic to surrounding tissues. Zinc oxide nanoparticles have bactericidal effects on both Gram-positive and Gram-negative bacteria (Arabi et al., 2012) and also effective against spores which are resistant to high temperature and high pressure (Rosi and Mirkin, 2005). When bacteria were treated with ZnO NP, there is a significant increase in its permeability affecting proper transport through the plasma membrane (Auffan et al., 2009) resulting in cell death. Antibacterial activity of ZnO NP depends on the surface area and concentration; whereas, the crystalline structure and particle shape have little effect (Arabi et al., 2012). But some other researchers found that, size is inversely proportional to the antibacterial property which means smaller the size of ZnO, better is the antibacterial activity (Shrivastava et al., 2007).

Nanoparticles have larger surface area available for interaction with the bacterial surface to enhance bactericidal effect than the large sized particles (Adams et al., 2006) because of its cytotoxicity to the microorganisms. Antibacterial effect of ZnO NP depends on concentration (Arabi et al., 2012). But still, the actual mechanism by which ZnO NP penetrate the bacterial cell wall is not fully understood. A number of authors have reported several mechanisms by which ZnO NP act against pathogenic bacteria. Raad et al. (2005) reported that nano materials release ions, which react with the thiol groups (−SH) of proteins present on the cell surface. These proteins protrude through the cell wall to allow the transport of nutrients. Zinc oxide nanoparticles inactivate the proteins, decreasing the membrane permeability and eventually causing the cellular death (Rajendran et al., 2010). Padmavathy and Vijayaraghavan (2008) reported that minerals in nano form also retard the bacterial adhesion and biofilm formation. Zinc oxide nanoparticles may also penetrate inside the bacterial cell and cause cell damage by interacting with phosphorus and sulfur containing compounds like DNA (Arabi et al., 2012). One more possible mechanism for antibiotic property of ZnO NP indicate that, microorganisms carry a negative charge while metal oxides carry a positive charge creating an "*electromagnetic*" attraction between the microbe and treated surface (Arabi et al., 2012). Once the contact is made, the microbe is oxidized and instantly dies. The nonspecific mode of action of NP against bacteria makes them ideal candidates as antimicrobial agents without risk of developing bacterial resistance (Arabi et al., 2012). Complete bacterial inhibition depends upon the concentrations of ZnO NP and on the number of bacterial cells. Thus, it is evident from the literature that ZnO NP have an excellent antibacterial properties and may be incorporated in animal feed as a growth promoting agent or to prevent the occurrence of diseases. In future, research should focus on utilizing nZnO as an alternative to conventional Zn sources in animal feed to reduce the use of in-feed antibiotic and also other benefits.

2.7. Toxicity of nZn

The potential hazard of high concentrations of nZn is still unknown and their toxicological data are rather uncommon. But still

Table 3
Antimicrobial activity of nano zinc (nZn).

Organisms affected; dose and particle size of nZn	Salient findings	References
Listeria monocytogenes; dose: 30 , 60, and 90 μg/mL	Concentration of zinc oxide nanoparticles (ZnO NP) is inversely proportional to growth of *L. monocytogenes*; nZn toxic to *L. monocytogenes*.	Arabi et al. (2012)
Pseudomonas aeruginosa and Escherichia coli (E. coli)	$Ag^{+} > Na^{+} > Zn^{2+} > Cu^{2+}$ is the order of antibacterial activity.	Top and Ülkü (2004)
E. Coli O157:H7; in stored food	Growth inhibition is directly proportional to concentrations of ZnO NP; distort and damage bacterial cell membrane, resulting in a leakage of intracellular contents and eventually the death of bacterial cells. Zinc oxide nanoparticles is a potential antibacterial agent in agricultural and food safety.	Liu et al. (2009)
L. monocytogenes, *Salmonella enteritidis*, *and E. Coli* O157:H7.	Zinc oxide nanoparticles is having significant antimicrobial activities against all 3 pathogens in growth media. Application of ZnO NP in food systems may be effective at inhibiting certain pathogens.	Jin et al. (2009)
Staphylococcus aureus (strain RN6390)	Zinc oxide nanoparticles has significantly higher antibacterial effects on *S. aureus*; antibacterial activity is inversely proportional to the size of the nZn; ZnO NP has a potential application as a bacteriostatic agent in visible light.	Jones et al. (2008)
P. aeruginosa, particle size: 10 to 20 nm	Bacterial attachment by electrostatic interaction, reactive oxygen species (ROS) generation, membrane disruption, and disturbance of permeability.	Feris et al. (2010)
Bacterium sp. (EMB4), particle size: 2 or 5 mm	Electrostatic interaction, morphological changes in the presence of nZn and non- nZn, increase in membrane permeability and ZnO accumulation in the cytoplasm.	Sinha et al. (2011)
Bacillus subtilis, particle size: 10 nm	Less toxic to Gram-positive organisms due to thicker peptidoglycan layer. Electrostatic interactions between NP (nanoparticles) and cell surface as the primary step towards nanotoxicity, followed by cell morphological changes, increase in membrane permeability thus leading to accumulation in the cell cytoplasm.	Sinha et al. (2011)
E. Coli, particle size 20 to 40 nm, 12 nm, 45 nm	Better bacteriocidal activity than bigger ZnO particles. The abrasiveness and the surface oxygen species of ZnO NP promote the bactericidal effects of ZnO NP.	Padmavathy and Vijayaraghavan (2008)
S. aureus, *E. Coli*, particle size: 60 to 75 nm.	Higher antibacterial activity was observed against *S. Aureus* than *E. Coli* both qualitatively as well as quantitatively.	Rajendran et al. (2010)
Vibrio fischeri, particle size: 50 to 70 nm	Zn formulations were very toxic to these organisms *in vitro*; minimum inhibitory concentration (MIC) for nZn is also very less as compared with other antibacterial preparations.	Heinlaan et al. (2008)
Anti-parasitic activities, larvicidal effects (*In vitro*)	The maximum efficacy was observed in nano ZnO against the *Rhipicephalus microplus*, *Pediculus humanus capitis*, and the larvae of *Anopheles subpictus*, *Culex quinquefasciatus*. Mortality of the parasites was 100% against *R. Microplus* (after 12 h), *P. humanus capitis* (after 6 h), lice (10 mg/L treated for 6 h). It is also having larvicidal effect against *A. Subpictus* and *C. quinquefasciatus*.	Kirthi et al. (2011)

the toxicity of Zn in food and feeds has been reported and presented in Table 4. Most of the toxicological studies have been done on rodents as *in vivo* models due to the similarity in biochemical and physiological pathways with human metabolism (Argmann et al., 2005). In Zn toxicity, pathological changes in the pancreas, kidney, liver, rumen, abomasum, small intestine and adrenal gland were observed in sheep (Allen et al., 1983). Liver, spleen, heart, pancreas and bone are the target organs of ZnO NP on oral exposure (Wang et al., 2008). In the histopathological examination, ZnO NP have dose and time dependent cytotoxicity and its mechanism is carried by oxidative stress, lipid peroxidation, cell membrane damage, and oxidative DNA damage (Lin et al., 2009; Najafzadeh et al., 2013). Zinc oxide nanoparticles induced toxicity in cells resulted in the production of free radicals causing oxidative injury, excitation of inflammation and cell death (Xia et al., 2008).

Toxic effects of the NP are size-dependent and nZn has been shown to be more toxic than micro-sized Zn at the same dose (Chen et al., 2007). Zinc oxide nanoparticles tend to accumulate in the liver tissues thereby causing the toxicity. Najafzadeh et al. (2013) reported mild liver toxicity (edema and degeneration in the hepatocytes) and severe renal damage (multifoacal interstitial nephritis in 75% of animals) in lambs because of nZn feeding at a dose of 20 mg/kg body weight orally for a period of 25 days. In mice, mortality was not observed even by feeding 20 or 120 nm ZnO at 1 g/kg body weight orally (Wang et al., 2008). Liver enzymes such as ALT and AST, ALP content in serum was increased in mice with ZnO NP treated group than the control groups (Sharma et al., 2012). The mechanism of toxicosis may be, as the nZn is much more active and can be rapidly transformed into respective ions in gastric juice. So large amounts of metal ions are generated and subsequently brought to liver and kidney for metabolism and excretion, which cause damage to hepatic and renal tissues (Chen et al., 2007). By feeding ZnO NP at a dose of 300 mg/kg orally to mice for 14 consecutive days, elevated ALT and ALP serum levels and pathological lesions in the liver were reported (Sharma et al., 2012). Cytotoxic effects of feeding ZnO NP include induced oxidative stress mediated DNA damage and increase in lipid peroxidation ultimately causing apoptosis (Sharma et al., 2012) and accumulation of ZnO NP in liver.

The toxicity of Zn is reported to be associated with the concentration of the free ion (Kasemets et al., 2009; Kool et al., 2011). But ZnO NP are likely to have remained as NP for longer duration,

Table 4
Toxicity reports of nano zinc supplementation.

Species	Dose	Organs affected	Toxicity	References
Mice	5 g/kg body weight	Liver, kidney	Inflammation in stomach, intestines, elevated ALT, ALP, and LDH in the nano Zn (nZn) group. Nano Zn supplementation is having less hepatotoxicity than micro Zn. Severe lesions in kidney on histopathological examination in the nZn group. Anaemia, hepatotoxic, renal toxic and also slight stomach and intestinal inflammation.	Wang et al. (2006)
Mice	20-nm and 120-nm ZnO powder at doses of 1-, 2-, 3-, 4-, 5-g/kg body weight	Stomach, liver heart and spleen	Zn was mainly retained in the bone, kidney and pancreas; increase in blood viscosity; 120-nm ZnO treated mice had dose dependant pathological damages in stomach, liver, heart and spleen; 20-nm ZnO displayed inverse dose dependant damages in liver, spleen and pancreas; liver, spleen, heart, pancreas and bone are the target organs of zinc oxide nanoparticles (ZnO NP) on oral exposure.	Wang et al. (2008)
Algae, crustaceans and fishes	–	Gene expression of metallothionin, heat shock protein, SOD	More toxic towards algae than crustaceans and fish. The toxicity is due to dissolved Zn^{2+} ions. Exposure to ZnO NP caused a significant up-regulation of superoxide dismutase (SOD), metallothionein (MT). Heat shock protein 70 was increased 2- to 4-fold indication substantial oxidative stress.	Wong et al. (2010)
Zebrafish	5 mg/L	Stomach, liver	The malondialdehyde levels in the liver was elevated and gut tissues exhibited oxidative effects after exposure.	Xiong et al. (2011)
Human	–	Gene expression of keratinocytes	Zinc oxide nanoparticles can produce ROS inducing oxidative stress. Antioxidant enzymes and SOD levels were significantly higher and glutathione levels were decreased in ZnO NP exposed cells; up-regulation of SOD genes by ZnO NP could increase the production of ROS and oxidative stress.	Lee et al. (2014)
Sheep	20 mg/kg body weight orally for 25 d	Liver and kidney	Alkaline phosphatase significantly decreased and creatinine level was significantly increased by ZnO NP. Cell swelling, eosinophilic necrosis of hepatocytes, and multifocal interstitial nephritis were also observed.	Najafzadeh et al. (2013)
Eisenia veneta (earthworm)	250 and 750 mg Zn/kg for 21 d.	Immune activity body Zn concentrations	Zinc oxide nanoparticles are less toxic than $ZnCl_2$. At 750 mg Zn/kg, reproduction declined by 50% when exposed to ZnO NP; but was almost completely inhibited by $ZnCl_2$. Immune activity was unaffected by ZnO NP but was suppressed by 20% when exposed to $ZnCl_2$. Nanoparticles can be taken up in particulate form.	Hooper et al. (2011)
Saccharomyces cerevisiae	–	Growth, recombinant microbial sensors	Nano and macro ZnO were of comparable toxicity. The toxicity was explained by soluble Zn ions.	Kasemets et al. (2009)
Daphnia magna	–	Reproduction	Toxicity is independent of particle size, coating of particles, aggregation of particles, the type of medium or the applied pre-treatment of the test dispersions.	Wiench et al. (2009)
D. magna	28 and 61 μg/L	Reproduction	Drop in number of juveniles per adult. Drop in reproductive performance from generation to generation. Elevated zinc accumulation in the 61 μg/L.	De Schamphelaere et al. (2004)
Folsomia candida	–	Reproduction	Toxicity of the Zn supplementation depends on the Zn ions released, not on the particle size of the Zn sources. Survival of *F. Candida* was not affected by particle size of ZnO. Reproduction was dose-dependently reduced with the Zn source.	Kool et al. (2011)

ALT = alanine aminotransferase; ALP = alkaline phosphatase; LDH = lactate dehydrogenase.

and thus are less toxic than the corresponding inorganic salts like $ZnCl_2$ (Hooper et al., 2011). The toxicity of Zn has been shown to be independent of particle size, coating of particles, aggregation of particles, the type of medium or the applied pre-treatment of the test dispersions (Wiench et al., 2009).

3. Summary

Role of Zn in the animal system is well realised and documented. But Zn from conventional sources is less available to the body and thus mostly excreted to the environment causing environmental

pollution. Nano Zn, as a substitute to the conventional Zn sources, can be a good alternative in livestock feeding. Apart from being highly bioavailable, reports have pointed out the growth promoting, antibacterial, immuno-modulatory and many other beneficial effects of nZn. This also serves all the purposes of the conventional Zn sources and helps in all the physiological functions. Thus, nano Zn may be used at lower doses in livestock feed to provide better results than the conventional Zn sources and indirectly prevents environmental contamination also. The toxicological studies provide mixed results in animal models. So, thorough and systematic studies are recommended for elucidating toxic effects, in any, dose fixation and also for economic production procedures to take nZn journey to logical conclusions.

Nano science is at its infancy in the field of mineral nutrition and further works are required in future to understand the effect of nano minerals, their site of absorption, mechanism of absorption, molecular basis of distribution and mode of action. The gene expression studies may also be designed as per the outcomes of different studies and can be compared with expression level of the conventional sources of nano minerals to know its effectiveness. Along with this, the possible toxicological effect in both ruminants and non-ruminants along with the toxic doses needs to be studied before they can be used in the rations. Further, research should be directed to find the optimum levels of nZn in ration that can provide better performance and economic benefits.

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