NANOMINERALS: A POTENTIAL SUBSTITUTE FOR ANTIBIOTICS IN GUT-ASSOCIATED PATHOGENIC INFECTIONS OF LIVESTOCK

D.T. PAL^{1*}, N. K. S. GOWDA¹ AND D. K. DEY¹

¹ICAR-National Institute of Animal Nutrition and Physiology, Bangalore- 560 030, Karnataka, India

Abstract

Diseases of the gastrointestinal (GI) tract or the gut could be bacterial, viral, fungal and/or parasitic such as protozoal, helminth etc. Out of all these causes, the bacterial diseases associated with the gut are predominant. Bacterial diseases of the gut not only implicate the functions of the gut, but its effects get manifested through disturbances in other physiological functions of the body as well. The most pertinent approach to ameliorate the gut associated bacterial infection are the use of antibiotics. Besides use as therapeutics, antibiotics are also used as growth promoters in poultry, and sometimes in pigs. Sustained and indiscriminate use of antibiotics have resulted in development of microbial resistance to antibiotics, and give rise to 'super-bugs', which are practically unmanageable at times. Moreover, the antibiotic residues are found in foods of the animal origin like milk, meat and eggs which cause a serious public health concern. These caveats have challenged the research community to explore novel antimicrobial agents. Nano materials as antimicrobials complementary to antibiotics are highly promising. Besides being highly bioavailable, nanominerals also facilitate in promoting growth in livestock, and imparting antimicrobial and immunomodulatory effects. The important antibacterial mode of action of the nanominerals are disruption of membrane integrity and/or its potential, production of reactive oxygen species, depletion of ATP production, denaturation of cytoplasmic ribosome subunit and disruption of protein translation. Metals and metal oxides nanoparticles such as silver (Ag), iron oxide (Fe₃O₄), titanium oxide (TiO₂), copper oxide (CuO), and zinc oxide (ZnO) nanoparticles are predominantly known to impart antimicrobial activity.

Key words: Antimicrobials, Gut health, Livestock, Nano minerals

Introduction

Since the past two decades, worldwide extensive research on both human and animal health revealed that the population of gut microbiome and their activity in the gut are responsible for the overall health and well-being of the host. A proper gut health is an indicator of good animal production (Kogut and Arsenault, 2016). Gut health includes several positive aspects of the gastrointestinal (GI) tract, such as the effective digestion and absorption of food, the absence of GI illness, normal and stable intestinal microbiota, effective immune status and a state of well-being. The GI barrier which lies in close proximity to the GI

microbiota is an important aspect in understanding the complex mechanisms that maintain gut health. An impairment in the GI barrier can increase the risk of developing infectious, inflammatory and functional GI diseases, as well as extraintestinal diseases such as immune-mediated and metabolic disorders. Actually, gut health comprises a healthy upper and lower GI tract, although the term might suggest that it is majorly restricted to the lower GI tract wherein the microbiome or the gut microbes reside in abundance (Bischoff, 2011). Therefore, gut health can be defined as "a steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium

*Corresponding Author

and where the welfare and performance of the animal is not constrained by intestinal dysfunction” (Celi *et al.*, 2017). Surprisingly, xenobiotic intervention rarely affects the host genome, however, the microbiome is readily affected by the changes in the diet, ingestion of antibiotics, and infection by pathogens, and other life events (Oakley *et al.*, 2014).

The higher prevalence of the diseases associated with the GI tract is attributed to the fact that the gastrointestinal tract has the most extensive exposed surface in the body and is constantly exposed to a wide variety of potentially harmful substances (Yegani and Korver, 2008). Bacteria and protozoa, which include *Salmonella*, *E. coli* and *Cryptosporidium* etc., can negatively affect the GI barrier. A breach in the GI epithelial lining opens up multiple gateways for other bacteria, toxins, and pathogens causing the animal to be susceptible to diseases affecting multiple organs, and subsequently deteriorating the overall health status of the host animal. Improvised methods of amelioration of the digestive tract diseases in combination with proper feeding strategies can improve animal health beyond the gut (Hunt *et al.*, 2002; Klein *et al.*, 2008).

In order to ameliorate the gut associated pathogenic infestations and normalise the animal's health (particularly those caused by pathogenic bacteria), diverse broad-spectrum antibiotics have been in use since decades. Moreover, it has been a common practise to include sub-therapeutic doses of antibiotics in animal diets to promote growth of the animals owing to their ability to increase feed efficiency or prevent diseases, especially prevalent in the poultry industry (Kogut and Arsenault, 2016). Antibiotics have a profound effect on the normal microbiota of the host, thereby upsetting the ecological balance of the gut (Oakley *et al.*, 2014). Deliberate and indiscriminate uses of antibiotics have resulted in development of

multi-drug resistant population of bacteria. Thus, antimicrobial resistance poses a substantial global health concern and there is critical need for alternate therapeutic options to combat infections. The indiscriminate use of antimicrobials is resulting in escalated incidences of antibiotic resistance and triggering epidemics of hyper-virulent pathogens among livestock. Moreover, the transfer of antibiotic residue to humans as well as other animals through consumption of foods and food products of animal origin poses a significant public health concern for the consumers of milk, meat and eggs. In this regard, nano minerals have the future to be used as an alternative of antibiotic growth promoters to overcome the above problems. Contrary to prebiotics and probiotics which have competitive effect on pathogenic microbes in gut, nano minerals have the direct effect on pathogenic microbes for efficient management of gut health. Peters *et al.* (2016) reported about the vivid spectrum of application of nanotechnology as a tool in the fields of biology, biotechnology, mineral nutrition, physiology, reproduction and pharmacology in animals. Metal nanoparticles (NPs) are among the most promising of these because they exhibit a strong antibacterial activity.

Antibacterial activity of metal and metal-oxide nanoparticles

Antibacterial activity of metal and metal-oxide nanoparticles (NPs) has been extensively reported by Hemeg (2017). The size of the particles and the large surface area relative to volume of metal and metal oxide NPs enables intimate interactions with microbial membranes, as well as surface function which help in developing more effective antibacterial agents. Moreover, nanoparticles with antibacterial activities have the potential to reduce or eliminate the evolution of more resistant bacteria because nanoparticles target multiple species of bacteria at the same time, thereby averting the development of resistant strains. It also has the capability to overcome drug resistance due

to their multi-functionality, as bacteria cannot develop multiple gene mutations simultaneously.

Over the last decade, there has been a remarkable focus globally on conventional as well as biogenic metallic nanoparticles as innovative tools for combating the high rates of antimicrobial resistance (Hemeg, 2017). In this regard, nanomaterials have shown promising results owing to their unique physical and chemical attributes (Pelgrift and Friedman, 2013; Beyth *et al.*, 2015). Several studies have pointed out the antibacterial action of the metal nanoparticles (Theivasanthi and Alagar, 2011; Hajipour *et al.*, 2012; Khurana *et al.*, 2016; Sportelli *et al.*, 2016; Patra and Baek, 2017).

Mechanisms of antibacterial activity of metal and metal-oxide nanoparticles

Several metal oxides in form of nanoparticles have been reported to exhibit marked antibacterial activity allowing efficient eradication of various bacterial strains. Each metal ion has its own sensitivity to different microorganisms (Ruddaraju *et al.*, 2020). Nano particles can alter the metabolic activity of bacteria by maintaining contact with bacterial cells via electrostatic interaction, Van der Waals forces, receptor-ligand and hydrophobic interactions (Choi *et al.*, 2017). Nano particles interact with the basic components of a bacterial cell causing oxidative stress, alterations in permeability and gene expression, disorders in electrolyte balance, protein deactivation and/or denaturation, and enzyme inhibition (Yang *et al.*, 2009). Most of the current research proposes oxidative stress, metal ion release, and non-oxidative mechanisms as critical aspects of action of the nano minerals for manifestation of its bactericidal effects.

Both nanoparticles and metal ions are absorbed through the cell membrane of microbes. Once within a bacterial cell, the production of reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions and

hydrogen peroxide inhibit DNA replication as well as amino acid synthesis. Moreover, it also damages the bacterial cell membranes via lipid peroxidation, compromising the membrane's semi permeability and repressing oxidative phosphorylation. AgO- and ZnO-nanoparticles have been reported to exert antibacterial activity by release of Ag ions and Zn ions that disrupt the membrane (Franci *et al.*, 2015; Sirelkhatim *et al.*, 2015; Dakal *et al.*, 2016; Duran *et al.*, 2016). Free Cu ions from Cu-containing nanoparticles and Mg nanoparticles also induce formation of ROS (Hemeg, 2017). The antibacterial action of Ag-nanoparticle is revealed by interaction of Ag ions with sulfhydryl groups in enzymes and other cellular constituents, making the latter dysfunctional. Ag ions also preclude cell wall synthesis in Gram-positive bacteria. Cu ions interact with amine and carboxyl groups on the surfaces of microbial cells, such as *Bacillus subtilis*. Au-nanominerals also result in bacterial membrane disruption (Yoon *et al.*, 2007). Inhibition of DNA replication and DNA degradation has also been reported with Cu-nanoparticles (Yoon *et al.*, 2007; Chatterjee *et al.*, 2014). Adsorption of nanominerals to bacterial cell is toxic to latter; with the positively charged nanoparticles being more toxic, which has been observed with TiO₂ and surface-modified Au-nanominerals (Wong *et al.*, 2015; Shamaila *et al.*, 2016). The Ag- and ZnO-nanoparticles also results in damage to membrane lipids and proteins of bacterial cell surface (Sirelkhatim *et al.*, 2015; Dakal *et al.*, 2016; Duran *et al.*, 2016). Ag ions inhibit cytochromes of the electron transport chain, and disrupt cellular transport systems (Pelgrift and Friedman, 2013). Loss of membrane potential and disruption of respiratory chain has also been observed with Au, Cu and NiO nanoparticles (Chatterjee *et al.*, 2014; Khashan *et al.*, 2016; Shamaila *et al.*, 2016). Nanominerals also affect the internalization and subcellular localization, resulting in toxicity. In the cytoplasm,

nanominerals exert microbicidal effects via oxidative stress and denaturation of the 30S ribosomal subunit, thereby impeding protein translation (Rai *et al.*, 2012; Duran *et al.*, 2016; Shamaila *et al.*, 2016). Furthermore, several researchers have reported that nanominerals comprising of Ag, Se, ZnO, TiO₂, Cd, Mg and Bi can disrupt bacterial biofilms. (Hernandez-Delgadillo *et al.*, 2012; Lellouche *et al.*, 2012; Lee *et al.*, 2014; Dakal *et al.*, 2016; Duran *et al.*, 2016; Guisbiers *et al.*, 2016). In a pictorial summary, the following anti-bacterial mechanisms occur through the application of nanominerals (Fig.1)

Fig. 1. Antimicrobial mechanisms of nanominerals

Copper nanoparticles as anti-microbial agent in gut health

In recent years, many studies have reported copper nanoparticles (Cu-NP) as a promising alternative to antibacterial reagents and a growth promoter. The use of Cunanoparticles has recently gained momentum due to their high electrical and melting points, low electrochemical migration behaviour, high bioavailability and relatively low cost of production (Tamilvanan *et al.*, 2014). Cu-NP, because of their high physical reactivity, could be used as an alternative for an effective health and growth promoter in much smaller doses than bulk minerals in animal feed (Gonzales-Eguia *et al.*, 2009; Joshua *et al.*, 2016; Scott *et al.*, 2016), thereby significantly reducing the excretion of these minerals into the environment. Moreover, Camacho-Flores

et al. (2015) reported Cu-NPs to be an effective antibacterial, antifungal and antiviral agent. Although the mechanism of action as an antibacterial agent has still not been explicitly described, reports have suggested that the mechanism of antibacterial activity of Cu-NP could be the same as that of silver nanoparticles (Rakhmetova *et al.*, 2010; Rajendran *et al.*, 2013). The antibacterial activity of Cu-NP depends on the physical characteristics of the nanomaterial. The size of the Cu-NP affects its toxicity by rapid and profound cellular uptake and translocation of the particles in the animal's body (Gatoo *et al.*, 2014). On the similar aspect,

Civardi *et al.* (2015) have reported that the small size of the Cu-NP can increase its uptake from the GI tract, hence, making them more effective than the bulk Cu mineral, even at lower doses.

The inorganic copper salts used for synthesis of nanoparticles highly affect the antimicrobial property of nanominerals. The crystalline Cu-NP has strong antimicrobial activity against both Gram-positive and Gram-negative bacteria (Scott *et al.*, 2018). The different shape, size and strong antimicrobial activity of Cu-NP have higher prospective in the field of biomedical sciences and food packaging (Shankar and Rhim, 2014). Copper nanoparticles due to their larger surface area are more effective antimicrobials as compared to organic and inorganic materials used in livestock health

(Zaboli *et al.*, 2013). It has been reported that the copper nanoparticle less than 100nm size can enter into the blood stream through GI tract, and then enter into various organs and tissues. In poultry, it has been attributed to enhancing the immunity by influencing the gut microbiota (Wang *et al.*, 2011; Thulasi *et al.*, 2013). The linoleic acid-capped Cu-NP have immense bactericidal action for *S. aureus*, *E. coli* and *Bacillus subtilis*, suggesting that Cu-NP can be used as effective growth inhibitors against various microorganisms (Das *et al.*, 2010), making them applicable in improving gut health in livestock. Rajasekaran and Santra (2015) demonstrated that the chitosan implanted with Cu-NP reduce gut bacteria such as *E. coli*, *Enterococcus faecalis*, *S. Aureus* and particularly, *Lactobacillus fermentum*, which could be used to minimise undesirable levels of microbial populations. The mechanism may be due to the dissipation of the cell membrane under the influence of nanoparticle accumulation or the generation of ROS by highly concentrated Cu²⁺ ions (Chatterjee *et al.*, 2014). Ramyadevi *et al.* (2012) reported that the Cu-NP is more toxic to bacteria than fungi. Moreover, the interactions of Cu-NP with the immune system are a key issue to guide the future use of Cu-NP in animal feed and medicine.

Zinc nanoparticles as anti-microbial agent in gut health

The importance of Zn in the animal system is well realised and explicitly documented. 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, immunomodulatory and many other beneficial effects of nano-Zn. Among metal nano particles, nano-zinc is the third highest globally produced nanometal after nano-SiO₂ and nano-TiO₂ (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). ZnO-NP were shown to have a wide range of antimicrobial activity against various microorganisms (Palanikumar *et al.*, 2014). Studies have already proved the dose dependant effect of ZnO-NP as antimicrobial and immunomodulatory agent by reducing the diarrhoea rate in piglets (Hongfu, 2008). It has been observed that the health status and immunity of the birds by supplementing nano-Zn to broiler diets at 0.06 mg/kg has improved significantly, as compared with the conventional dose of 15 mg/kg of organic and inorganic Zn with the basal diet (Sahoo *et al.*, 2014a,b). Further, Hongfu (2008) has reported that 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. Hence, this establishes the fact that ZnO-NP are immensely effective antibacterial agents even at a very low concentration, compared to the conventional ZnO.

Selenium nanoparticles as anti-microbial agent for gut health

Selenium nanoparticles (Se-NP) have been reported to possess potent antibacterial activity (Hirai *et al.*, 2010; Yip *et al.*, 2014). Se-NP when fed to broilers at a dose rate of 0.3 ppm and 0.5 ppm compared to both organic and inorganic selenium sources showed more efficacy and better performance in the treated group compared to control (Bagheri *et al.*, 2015; Boostani *et al.*, 2015; Selim *et al.*, 2015). Se-NPs cross the membrane barriers of the bacteria imparting their antibacterial activity. Huang *et al.* (2016) reported that nano-selenium particle has efficient antibacterial and bactericidal activities, proving that these are good antimicrobial alternatives for the development of antimicrobial agents. Furthermore, they also stated that acetylcholine

hybrids of Se-NP have the ability to combine with the acetylcholine receptor on the bacteria cell membrane and increase the permeability of cell membranes. This causes membrane disruption and leads to the leakage of the cytoplasm, allowing nanoparticles to subsequently invade bacterial cells and disrupt the DNA structure. Therefore, the acetylcholine-conjugated Se-nanoparticles have enhanced antibacterial activity. Quercetin and acetylcholine conjugated Se-nano particles significantly decrease the viability of *E. coli* and *S. aureus* cells over time. *E. coli* and *S. Aureus* when treated with Se- nanoparticles showed 60% reduction in the viability of both Gram-positive and Gram-negative bacteria. This study results reveal a higher potency of antibacterial activities of quercetin and acetylcholine conjugated Se-nanoparticles. Besides this, the latter exhibit a synergistically enhanced antibacterial performance against the multi-drug resistant superbugs. Huang *et al.* (2016) reported that high antibacterial activity of Se-NPs is due to compromised bacterial cell membranes' integrity, by increased intracellular production of ROS, and/or disruption of the bacterial DNA structure upon entry into the cell. Se-NPs also restrain growth and biofilm formation of *E. coli* and *S. Aureus* (Guisbiers *et al.*, 2016).

Silver nanoparticles as anti-microbials for gut health

Silver has a strong antimicrobial potential, and has been used since the ancient times. Silver in the form of various compounds and bhasmas (Ash) have been used in Ayurveda to treat several bacterial infections since time immemorial. With time, as several pathogenic bacteria are developing antibiotic resistance, silver nanoparticles are the new hope to combat them. Silver nanoparticles have been widely used as an effective antimicrobial agent against bacteria, fungi and viruses (Rai *et al.*, 2009).

Although the mechanism of action of Ag-nanoparticle is still not clear,

Ag-nanoparticles of smaller diameter have a superior antimicrobial effect than those of their counterparts with larger diameter (Panacek *et al.*, 2006). Silver ions are positively charged. This physical property of the Ag ion is crucial for its antimicrobial activity by virtue of the electrostatic interaction between negative charged cell membrane of microorganism and positive charged nanoparticles (Hamouda *et al.*, 2000; Dibrov *et al.*, 2002). Ag-nanoparticles increase the membrane permeability and inactivate the respiratory chain in Gram-negative bacteria like *E. coli* (Sondi and Salopek-Sondi, 2004; Beyth *et al.*, 2010). Choi *et al.* (2008) revealed that the Ag ion which has an affinity for sulphur and nitrogen. This can inhibit and/or disrupt protein structure by binding to thiol and amino groups. Many earlier studies have further reported that silver nanominerals are photocatalytic and can induce production of ROS (Carlson *et al.*, 2008; Piao *et al.*, 2011; Ashok Kumar *et al.*, 2014; Ninganagouda *et al.*, 2014). Studies by Shahverdi *et al.* (2007) and Khurana *et al.* (2014) indicated that Ag-nanominerals also have synergistic antibacterial effects both on Gram-positive and Gram-negative bacteria when administered in combination with antibiotics. In yet another mechanism of antibacterial activity exhibited by Ag-nanoparticles is to bind to the thiol groups in enzymes, generating reactive oxygen species (ROS), and disrupting the conserved bacterial respiratory chain in different kinds of bacteria. In addition, Agions can also interact with microbial DNA, thereby inhibiting growth by obstructing DNA replication and cell division (Pineda *et al.*, 2012; Dakal *et al.*, 2016; Duran *et al.*, 2016). Fondevila *et al.* (2009) have reported extensively about the antibacterial activity of silver nanoparticles in chickens and pigs. Besides demonstrating a significant antibacterial property, Gholami-Ahangaran *et al.* (2013) demonstrated that silver nanoparticles reduced the effects of aflatoxin

on the growth and performance indices in broiler chickens suffering from experimental aflatoxicosis. While the supplementation of silver with a conventional diet had no effect on performance, however, the addition of silver nanoparticles to diet containing 3 ppm aflatoxin significantly increased the cumulative body-weight gain, cumulative feed consumption, and decreased the feed consumption ratio (FCR) in

the last two weeks of the experimental period. The study, therefore concluded that supplement of silver nanoparticles to diet containing aflatoxin could diminish the inhibitory effects of aflatoxin. Chauke and Siebrits (2012) in one of their study replaced an antibiotic against coccidiosis with silver nanoparticles and found effective. The activities of the metal and metal oxide nanoparticles are summarised in the table 1.

Table 1. Nano minerals and their effects on microbes

Metal nano-particles	Microbes affected	Effect on microbes	Reference
Cu	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , <i>Micrococcus luteus</i> , <i>Klebsiella pneumoniae</i> and <i>Pseudomonas aeruginosa</i>	Inhibits the growth, DNA replication, induce membrane damage and generation of ROS	Fang <i>et al.</i> , 2007; Raffi <i>et al.</i> , 2010; Applerot <i>et al.</i> , 2012; Pramanik <i>et al.</i> , 2012; Ramyadevi <i>et al.</i> , 2012; Shobha <i>et al.</i> , 2014
Zn	<i>L. monocytogenes</i>	Inhibits the growth and toxic to bacteria.	Arabi <i>et al.</i> , 2012
Zn	<i>Salmonella</i>	Membrane disruption and ROS production	Jin <i>et al.</i> , 2009
Zn	<i>Klebsiella pneumonia</i>	Membrane disruption and ROS production	Reddy <i>et al.</i> , 2014
Zn	<i>Streptococcus</i>	Membrane disruption and ROS production	Kasraei <i>et al.</i> , 2014
Zn	<i>E. Coli</i>	Growth inhibition, damage bacterial cell membrane, leakage of intracellular contents and eventually the death of bacterial cells	Liu <i>et al.</i> , 2009; Liu <i>et al.</i> , 2009; Rajendran <i>et al.</i> , 2010
Zn	<i>Staphylococcus aureus</i>	Higher antibacterial effects	Jones <i>et al.</i> , 2008; Rajendran <i>et al.</i> , 2010
Zn	<i>P. aeruginosa</i>	Generation of ROS, disruption of membrane permeability	Feris <i>et al.</i> , 2010
Zn	<i>Bacillus subtilis</i>	Electrostatic interactions, morphological changes in the cell, increase in membrane permeability	Sinha <i>et al.</i> , 2011
Se	<i>E. coli</i> and <i>S. aureus</i>	Inhibition of growth, directly kill bacteria by changing cell permeability, attach to the bacterial cell wall causing irreversible damage to the membrane	Hirai <i>et al.</i> , 2010; Huang <i>et al.</i> , 2016
Se	<i>S. aureus</i>	Inhibition of growth	Tran and Webster, 2011
Ag	<i>S. aureus</i> and <i>Pseudomonas aeruginosa</i>	Generation of ROS, disrupting bacterial respiratory chain	Sondi and Salopek-Sondi, 2004; Li <i>et al.</i> , 2005
Fe	<i>Pseudomonas aeruginosa</i>	Prevention of biofilm formation	Park <i>et al.</i> , 2011
Al	<i>E. coli</i>	Perforation and membrane disorganization, leading to cell death	Ansari <i>et al.</i> , 2014
Mg	<i>E. coli</i> and <i>S. aureus</i>	Prevent biofilm formation, inducing ROS, directly inhibit essential enzymes of the bacteria	Lellouche <i>et al.</i> , 2009; Blecher <i>et al.</i> , 2011

Nanominerals as growth-promoting agents

Because of the 'nano' size and higher bioavailability, metal nanoparticles are also used as feed additives to enhance growth, performance and production in livestock. These also serve as a potent replacement of the antibiotic-based feed-additives which are used as growth promoters. Mishra *et al.* (2014) have reported that zinc oxide nanoparticles enhance growth performance, improve feed utility and provide economic benefits in weaning piglets and poultry. It has been found that in ruminants, large doses of ZnO nanoparticles cannot act as growth promoters, however, Hongfu (2008) has demonstrated that feeding a dose rate up to 3,000 mg/kg feed have proved to be promoting growth in pigs. In yet another experiment, chromium nanoparticles were fed to poultry at a dose rate of 500 mcg/kg Cr³⁺. It not only affected the protein content of breast and thigh muscle positively; at the same time, it lowered the serum cholesterol levels too. It also improved average daily gain and feed efficiency of the broilers in the experimental group fed with chromium nanoparticles (Zha *et al.*, 2009). Copper has been reported to be added as feed-additive in order to promote animal growth performance in addition to its antimicrobial properties (Solaiman *et al.*, 2001; Zhao *et al.*, 2007). They have demonstrated that copper nanoparticles could improve piglet's energy and crude fat digestion through the augmentation of lipase and phospholipase A activity in the small intestine compared to a basal diet supplemented with CuSO₄. These piglets also experienced an increase in daily weight gain, metabolic rates as well as immune capacity. Scott *et al.* (2018) suggested that Cu-NP can allow more nutrients to be digested in the GIT, consequently improving the growth of chickens. In an experiment by Vijayakumar and Balakrishnan (2014), broiler birds fed with nano-form of calcium phosphate by replacing up to 50% requirement of dicalcium phosphate (DCP),

showed a best feed conversion ratio of 1.39 (± 0.02) and differed significantly from the control groups.

Conclusion

Nanotechnology thus confers an assurance as well as vast opportunity to attain specific target action by subtle modifications at a nano-scale. NPs are now being considered as a viable alternative to antibiotics due to their bactericidal, growth promoting and immune-potentiating properties. Metallic nanoparticles when used with the existing antibiotics for bacterial infections work synergistically by lowering the anti-biotics dosage to be administered, thereby minimizing toxicity as well as reducing the probability of development of resistance. Metal nanoparticles are advantageous over conventional systems of antibiotic therapy as they enhance therapeutic capacity by preventing microbial resistance. Further, metal nanoparticles have the capability to overcome drug resistance due to their multi-functionality, as bacteria cannot develop multiple gene mutations simultaneously. Apart from being highly bioavailable, using nanominerals as feed additives in livestock improve growth performance, and reduce the pathogen load to improve health and reduce the excretion of metals into the environment. Their small size and large surface-to-volume ratio make metal nanoparticles biologically more active than inorganic salts. Besides that, being highly bioavailable, these are required at a very low concentration compared to the conventional mineral salts, either as feed formulations or as therapeutics. Moreover, the most prominent and promising advantage is that there is no presence of antibiotic residues in foods of animal origin after using metal and/or metal oxide nanoparticles for antibacterial activity in the host animal. This fact is of stupendous significance when there is a global public

health concern with regard to presence of antibiotic residues in food. Therefore, with the most concerning issue of microorganism resistance to antibiotics, developing new nanoparticles using metal ions or metal oxides

is of great theoretical and practical concern to improve the gut health in livestock.

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