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Nano Copper in Poultry Nutrition: Potential Effects and Future Prospects-A Review

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ABSTRACT

Copper (Cu), an essential trace element plays a key role in maintenance, productionand health of birds. Hence, supplementation of Cu has become a common practice in poultry diets. However, thebioavailability of Cu from feed ingredients as well as from its inorganic saltsislow. Thus, strategies to improve bioavailability of Cu from its conventional sources for use in poultry diets assume significance. In this context, nano-technology has been demonstrated to be beneficial in various biological fields including poultry nutrition. Nano Cu (NCu) has additional novel properties (size, shape, concentration, surface charge and reactivity) compared to its conventionalinorganic form. The improved nutrients utilization and growth performance of birds was observed upon dietary inclusion of NCu. This effect was attributed to better gut health due to antimicrobial properties of NCu and also due to better expression of hypothalamic appetite regulating genes and dietary energy and fat utilization.The metabolizability of nutrients and growth performance of birds was improved or comparable at reduced NCulevel in the diets.Further, reduced Cu interaction withphytic acid, iron and zinc, increased Cu deposition in the tissues and reduced Cu excretion due to NCu inclusion in the poultry dietswereobserved. However, suppressed immune response and antioxidant statuswas reported due to relatively higher dose of NCuin poultry diets, due to inappropriate stimulation of immune system or over production of reactive oxygen species due to nanoparticles in the body.Due to numerous advantages, the application of NCu in poultry nutrition will continue to develop and further studies are required to fulfil the knowledge gap in physicochemical characteristicsincluding toxicityof nanoparticles in poultry.

KEYWORDS: Bioavailability, Copper, Nanoparticles, Poultry

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INTRODUCTION

Trace mineral nutrition of livestock and poultry received less attention due to their relatively low economic importance since it represents less than 0.5% of total feed cost. However, the environmental safety is an emerging concern increasing day-to-dayin parallel with the increasinginterest in the use of trace mineralsparticularly copper (Cu) and zinc (Zn)in poultry nutrition similar to nitrogen and phosphorus. Much has been known about theutilization, efficiency and excretion of N and P in poultry nutrition, whereas the lack ofsufficient information about Cuutilization in the diets and its excretion to the environment is still a concern.

The role of Cu in the maintenance, growth, production, reproduction and health of animals and birdsas an essential trace mineral is well recognized. Cu is an indispensable part of over200 important enzymessuch as cytochrome oxidase C and superoxide dismutase in the body (Wu et al., 2015). However, the Cu content of conventionally used feed resources is low and its bioavailability in such feed ingredients is less than 20% (Leeson, 2009). Therefore, additional Cu is necessary in the diets to meet the requirements for optimum growth and production of poultry and other livestock species. Exclusive Cu sources are commercially available in the form of inorganic salts of sulphate, chloride, oxide and carbonate. However, the Cu bioavailability from such inorganic sourcesis alsopoor (Scott et al., 2018) that resulting in Cu deficiency with more Cu excretion from the body and environmental pollution.Generally, the agriculture landwhere the poultry dropping is applied as soil fertilizer contains 660% Zn and 560% Cu over the crop requirements for optimal growth(Dozier et al., 2003).

The poor bioavailability of Cu leading to huge quantity of Cu excretion and environment contamination resultsin toxicity for plants and grazing animalsandencourages unfavorable microorganisms' growth in soil and water (Ferket et al., 2002).European Union has adopted a regulation to limit the Cu level in poultry diet that should not exceed 25 ppm to ensuretheenvironmental safety(EFSA, 2016).This emphasizes the need for interventions to enhancethe Cu utilization, efficiency and reduce its excretion while using conventional Cu sources in poultry diets.

Recently, nano-technology, the commercial application of nano-sized minerals has been in use in various fields such as biology, biotechnology, physiology, reproduction, pharmacology and livestock nutrition (Peters et al., 2016). Nanoparticle refers to a particle size of 1 to 100 nmdiameter that exhibit novel properties such as small size, greater specific surface area and activity, high catalytic efficiency and stronger absorbing ability that are different from conventional bulkparticles (Tamilvanan et al., 2014),greater bioavailability (Sharif et al., 2020) and less antagonism tendency with other minerals (Hefnawy and El-khaiat, 2015)which enable Cu atoms to directly reach target cells (Pineda et al., 2013).Thus, use of nano copper (NCu) has drawnmuch attention of nutritionists due to itspotential in animal nutrition (Tamilvanan et al., 2014; Sharif, et al., 2020).

The NCuin poultry diets increase the availability of nutrients and enhances their absorption from GIT resulting in beneficialeffects on growthperformance, nutrientsutilization and health, reduce excretion and contribute to environmental safety (Prescott and Baggot, 1993).In this review, the potential effect of NCu as an alternative Cu source in poultry dietonproduction performance, nutrients utilization, and immune response is discussed. The possibility of using NCu as an alternative growth-promoting supplement as well as to draw a map for the next steps of NCu application in poultry nutrition is also attempted.

BIOAVAILABILITYOF COPPER FROM DIFFERENT SOURCES

The feed ingredients arelower in Cu content on the one hand, and its variable bioavailability due to differences in agronomic conditions, practices and processing (NRC, 1994) on the other hand, are the major issues causing 80% of dietary Cu voided in the excreta of poultry. The dietary ingredients'Cu lower bioavailability may be due to greaterbinding tendency rate of Cu with phytate (Pang and Applegate, 2006) that forms an insoluble complex. The Cu in animal proteins seems to be even more variable in bioavailability. The Cu content in pig liver is totally unavailable for poultry and Cu deficiency in cats is reported when fed with porcine liver as a sole source of Cu (Leeson, 2009). To reduce Cu supplementation in the diet without compromising the production performance, knowledge about the bioavailability of Cu sources is essential.

Additional Cu is being supplemented in the diet from inorganic salts (such as sulphate, carbonate, chloride and oxides) so that to meet the requirement for optimal growth and production of poultry. These salts are broken down, minerals are released in free ionforms and then absorbed in the digestive tract of birds (Lyons and Jacques, 1998). However, free ions are very reactive and can form unavailable complexes with other dietary molecules and minerals, or making them difficult to absorb. As a result of the poor bioavailability of conventional Cu sources (Table 1) in feed, nutritionists are concerned about Cu utilization efficiency. Copper sulphate ($CuSO_4$) is usually considered as a standard to compare other Cu sources and the Cu concentration in the liver is directly correlated to the bioavailability of the subjected Cu source (Ledoux et al., 1991).

Table 1. Cu bioavailability from different Cu salts and feed sources in relation to $CuSO_4$

No.	Source / form of Cu	Species	Relative bioavailability (%)	Reference
		Cu bioavailability from different exclusive Cu salts		
1	Copper sulphate	Broilers	100	Byrne and Murphy (2022)
2	Tribasic copper chloride	Layers	107.4	Kim et al. (2016)
3	Cupric chloride, tribasic	Poultry	70–134	Byrne and Murphy (2022)
4	Copper oxide	Sheep	81	Byrne and Murphy (2022)
5	Copper oxide	Broilers	92.5	Baker et al. (1991)
6	Copper carbonate	Cattle	86	Byrne and Murphy (2022)
7	Copper carbonate	Sheep	97	Balakrishnan (2010)
8	Cupric acetate	Broiler	93–188	Byrne and Murphy (2022)
9	Copper chloride	Broilers	115	Balakrishnan (2010)
10	Cupric chloride	Poultry	106–110	Byrne and Murphy (2022)
11	Copper nitrate	Broilers	50	Balakrishnan (2010)
12	Copper methionine	Broiler	117	Wenet al. (2019)
13	Cu-Lysine	Broilers	115.5	Baker et al. (1991)
14	Organic Cu	Poultry	88-144	Lim and Paik (2006)
15	Copper proteinate	Poultry	79–111	Byrne and Murphy (2022)
		Cu bioavailability from feed resources		
1	Freeze-dried liver	Broilers	116	Aoyagi and Baker(1993)
2	Broiler by-product	Broilers	93	
3	Corn gluten	Broilers	48	
4	De-hulled soybean meal	Broilers	38	
5	Cotton seed meal	Broilers	41	
6	Peanut hulls	Broilers	44	
7	Soyabean	Broilers	40	Pang and Applegate (2006)
8	Groundnut cake	Broilers	50	

PHYSICO-CHEMICAL CHARACTERISTICS OF NANO COPPER

Copper nanoparticles have UV-visible sensitivity as well as catalytic, electrical, thermal, and antibacterial capabilities due to their huge surface-to-volume ratio and having many atoms on the surface (Wang, 2000).The biological activities of NCu are directly dependent on their physical and chemical properties of the particles such as size, shape, concentration, surface, charge and reactivity. However, nanoparticles' characteristics can be varied depending on the nanoparticle preparation technique and also the stage of formation.

The NCu characterization includes the electronic energy level, electron affinity, electronic transitions, magnetic properties, phase transition temperature, melting point and affinity for polymers, biological and organic molecules as a function of surface area change (Din and Rehan, 2017).The NCu's functional activities such as chemical, catalytic, and biological effects, are highly reliant on the nano-metals' particle size. The NCu can be characterized bydifferent methods such as ultraviolet-visible absorption or transmission electron microscopy for fine powders, atomic force microscopy and infrared spectroscopy. Other convenient methods of characterizing NCu are dynamic light scattering, X-ray scattering at small angles and ultraviolet-visible spectroscopy.

The physicochemical properties of NCu such as particulate core, particle surface oxidation state, particle surface charge, singlet and agglomerate sizes in relevant carriers, shape, solubility and surface area are still not thoroughly understood. These characteristics influence the nano-material dose delivered to the target organs. Furthermore, when NCu enters the body and during transportation, the surface properties are expected to change. Shankar and Rhim (2014) reported that the type of Cu salt employed for NCu synthesis has a significant impact on the morphological properties of NCu. The strong antimicrobial activity of NCu against both Gram-positive and Gram-negative bacteria were reported (Shankar and Rhim, 2014; *Abd El*-Ghany et al., 2021).

The biological system's response to nanoparticles in respect of distribution and eliminationof the material is dependent on the size and surface area of nano-materials. The size of the NCuis a major challenge for the scientific community to achieve appropriate physical and chemical characterization of nanoparticles and *in vivo* response.Surface characteristics, clearance and biocompatibility agglomeration of nanoparticles also can influence cellular uptake in the body (Shi et al., 2012). Due to the inflammatory influx of cells that have higher oxidation and cytotoxic potential, NCu can be more rapidly cleared from the biological system (Shi et al., 2012).

NUTRITIONAL AND PHYSIOLOGICAL CHARACTERISTICS OF NANO COPPER

The nano sized particles of Cu can enhance its uptake from the GIT, make it more effective than the conventional form, interact more efficiently with organic and inorganic components of diet due to their larger surface area and presence of more number of atoms on the surface (Zaboli et al., 2013).NCu are capable to cross the small intestine and distribute into the blood, brain, heart, kidney, spleen, liver and intestine (Hillyer and Albrecht, 2001). The materials ability to cross the epithelial barriers, its cellular uptake, cytotoxicity and bio-distribution also are largely dependent on the nanoparticles' solubility and protein binding capacity (Hagan, 1996). The nanoparticles are absorbed in to the duodenal enterocytes (Patra and Lalhriatpuii, 2019)by a variety of pathways including passive diffusion across the mucosal cells, active transport mechanisms such as phagocytosis or pinocytosis and intercellular transfer (Hagan, 1996).The smaller (2 nm) nanoparticles are filtrated out through glomerular filtration by kidneys, whereas bigger particles (40 nm) are retained in the Kupffer cellsin the liver (Sadauska et al., 2007).The way nanoparticles enter the cell has an importantimpact on how they affect biological processes.

NCu has been demonstrated to improve growth performance, production, reproduction, feed utilisation and efficiency in chicken, fish, and piglets when compared to the bulk form of $CuSO_4$(Gonzales et al., 2009; Mroczek et al., 2015). The improvedproductive performance is attributed to the better bioavailability and antibacterial properties of NCu that are stronger as compared to conventional form of $CuSO_4$(Usmanet al., 2013),while others concludeto be due togreater effect on energy and fat digestibility (Gonzales et al., 2009). Along with antimicrobial properties of NCu, greater anthelminthic effect is also reported in comparison to its conventional form of $CuSO_4$(Ramesh, 2014). Furthermore, some studies found that NCu dietary supplementation increase animal SOD activity (Refaie et al., 2015) that can lead to oxidative and immune suppression.

NANO COPPER TOXICITY

In an experiment, NCu (25 nm particle size) induced toxicological effects on kidney, liver and spleen in mice (Chen et al., 2005).The cytotoxicity induced by NCu may be due to increased Cu reactivity, porosity and high affinity ions level in different tissues and organs. Because of their small size, NPs able to penetrate cell and organelle membranes, block ion channels, hinder enzyme proteins, and interact with DNA resulting in DNA fragmentation which stops the vital functions of cells (Ognik et al., 2016a). The NCu are dissolved in acidic conditions such as in the gastric phase but not in the small intestine and NCu aggregate within the small intestine causing the toxic effect on enterocytes and can cross the intestinal wall causing toxicities in brain, heart, kidney, spleen and liver(De Jong et al., 2019).

POTENTIAL EFFECT OF NANO COPPER ON POULTRY PRODUCTION

Production performance

The NCu has better effect on growth performance and productivity in poultry (Samanta et al., 2011; Hefnawyand El-khaiat, 2015; Michalak et al., 2022). The improved birds' performance was due to stronger antimicrobial properties of NCuthat improve intestinal health (Nollet et al., 2007; Usman et al., 2013; Wang et al., 2014) and better effect on growth hormone axis and hypothalamic appetite regulating genes expression (Zhou et al., 1994).The Cu effect on growth performance is also associated with the lipase activity, growth hormone secretion and post absorptive lipid metabolism involved genes expression (Espinosa and Stein, 2021).

Studies indicated that NCu either improves growth performance at comparable doses (Mroczek et al., 2015; Aminullah et al., 2022) or maintains it at a reduced supplementation level (Ramesh, 2014; Sawosz et al.,2018; Aminullah et al., 2021; Aminullah et al., 2022a) in poultry in comparisonto conventional form of $CuSO_4$.However, there is still a literature gap on the potential effect of NCu on growth performance and other physiological effects in the systems. Further, it is difficult to compare different reports of the potential effects of NCu as the absorption and metabolism of NCuare dependent on physicochemical properties, method of administration and dose of nanoparticles (Lee et al., 2016).

The better effects of NCu are attributed to its novel properties and improved bioavailability (Mroczek et al., 2015; Sharif et al., 2020; Aminullah et al., 2021; Aminullah et al., 2022a) as compared to bulk form of $CuSO_4$.The potential effect of NCu on the production performance of poultry is summarized in Table 2.Due to NCu in the diet, reduced feed intake was observed in several studies without affecting the feed conversion ratio (FCR). This was attributed to improved feed efficiency and energy utilisation (Patra and Lalhriatpuii, 2019) due to greater effect of NCu that is associated with the novel properties (Wang, 2000) and better bioavailability of Cu nanoparticles (Tamilvanan et al., 2014). The improved activity of cytochrome oxidase in the presence of NCu playsan important role in production of cellular adenosine triphosphate and energy metabolism (Hill et al., 2000) and also associated with the improved feed energy utilization. The ability of nanoparticles to effectively penetrate cell membrane barriers, particularly those of intestinal endothelial cells, and rapidlyget distributed independent of blood circulation, also can contribute to better poultry production performance(Anjum et al., 2016).

Nutrients utilization and metabolizability

Copper supplementation has a positive impact on gut health, reduces pathogen load, improves' enzyme activities and nutrient utilization, and growth performance in poultry that are associated to the improved activities of digestive enzymes such as protease, amylase and lipase due to NCu (Luo and Dove, 1996). From other hand, the adverse effect of excess Cu accumulation in the liver leads to increased Cu concentration in droppingsand negatively affecting nutrient utilizationis also been reported (Yang et al., 2018).Therefore, lower Cu supplementation levels from a greater bioavailable sourceare suggested to improve the nutrients' metabolizability and prevent the possible adverse effect.

The improved nutrients utilization in poultry that was attributed to the greater bioavailability (Tamilvanan et al., 2014; Sharif et al., 2020) and stronger antimicrobial activities of NCu (Shankar and Rhim, 2014) as compared to bulk form of $CuSO_4$. The antimicrobial properties ensure improved gut health, reduced pathogen load (Michalak et al., 2022), leading tobetter nutrient utilization and growth performance in poultry (Samanta et al., 2011; Usman et al., 2013; Hefnawy and El-khaiat, 2015).Whereas, other researchers have attributed to the enhanced energy metabolizability due to NCu in the diet (Gonzales et al., 2009).It is also reported that the improved fat utilisation is associated with the improved lipase and phospholipase activities in the small intestine due to Cu supplementation (Das et al., 2010). The reduced feed intake without affecting the egg production due to NCu at 75% required level in Swarnadhara breeder hens was due to improved dietary energy and nutrient utilisation (Aminullah et al., 2021). The potential effect of NCu on nutrient metabolizability is summarised in Table 3.

Mineral balance and tissue deposition

Generally, large proportion of trace minerals are excreted resulting in environmental accumulation. Cu bind with phytic acid forming an insoluble Cu-phytate complex in the gut leading to calcium and phosphorus imbalances in the body (Maenz et al.,1999). Alteration in gut normal flora population due to antibacterial characteristics of NCu leads to reduction in phytase producing bacteria, lowering phytase activity and mineral solubility (Zhang et al., 2017).

Cu and Zn have an antagonistic interaction because both minerals bind to the same protein for absorption from the GIT (Ognik et al., 2019). Similarly, higher level of Cu is reported to induce Fe-Cu antagonism, resulting in reduced Fe absorption in the intestine (Suttle, 2010). The less interaction or antagonistic tendency of NCu as compared to higher level of the conventional form of $CuSO_4$ was reported (Hefnawy and El-khaiat, 2015).The Cu excretion can be reduced proportionally to the NCu inclusion level in the diet (Aminullah et al., 2022c).

The Cu accumulation in liver and muscle was also significantly ($p<0.05$) improved (Aminullah et al., 2022c). The bioavailability of Cubased on its concentration in the liver as result of NCu incorporation level in the diet was greater ($p>0.05$) (from 136to199%) in comparison to 100% conventional form of $CuSO_4$(Aminullah et al., 2022c). The result of the study confirmed the greater bioavailability of NCu (Patra and Lalhriatpuii, 2019;Sharif et al., 2020) in comparison to its bulk form of $CuSO_4$.The increased Cu content in both liver and muscle proportional to thence inclusion level in the diets was also reported by Ognik et al. (2019). Many researchers reported reduced Cu excretion proportionally to NCu inclusion level in the diet of poultry (Nollet et al., 2007; Gonzales et al., 2009; Sawosz et al.,2018; Aminullah et al., 2022c).

The uptake of NCu can occur through mucosal cell membranesby active transport mechanisms (Hagan, 1996). After absorption in the GIT, NCu enter the bloodstream and be retained in different organs and/or be rapidly distributed independent of the blood circulation (Anjum et al., 2016).As the absorption and metabolism of NCu are dependent on their physicochemical properties, different mechanisms of NCu absorption and distribution that differ from the normal form of $CuSO_4$ may explain its effective utilization, tissue deposition, and hence reduced excretion(Lee et al., 2016).The effect of NCu on minerals balance in the body and tissue deposition is summarized in Table 4.

Immune response and antioxidant status

Immune system is the key element indicating health status of poultry.The biological functions of

Cu are associated with its role in the active site of various metallo-enzymes such as cytochrome oxidase, superoxide dismutase (SOD), lysyl oxidase, dopamine hydroxylase and tyrosinase (Makarski and Zadura, 2006). In addition, white blood cell development also needs sufficient quantity of Cu (Sharma et al., 2005). Increasing the content of low-molecular and enzymatic antioxidants in poultry meat may significantly reduce undesirable oxidation processes, and thus improve the quality of the product and extend its shelf-life, which is important for the consumer.

Studies revealed that NCu can affect the immune status of biological system (Michalak et al., 2022), but the impacts might be varied depending on the supplemental dose. Ognik et al. (2017) reported improved antioxidant status in broilers due to NCu supplementation at 54% exceeding NRC (1994) recommended level in the diet, while at +96% versus the NRC recommendation, a decrease in the level of glutathione, glutathione disulfide and an increase in superoxide dismutase, catalase and ceruloplasmin activity and in lipid hydroperoxide content were observed.

The adverse effect of relatively higher dose of NCu on immune response and antioxidant status in chicken was reported (Morsy et al., 2021; Aminullah et al.,2022b) (Table 5).Inadequate stimulationof immunological function with nanoparticles may result in suppression of immune response (Shannahan and Brown, 2014). The immune system is reacting to metallic nanoparticles (Luo et al., 2015) leading to pro-inflammatory cytokinesactivation and over production of reactive oxygen species (ROS) leading to immune system imbalance (Najafi-Hajivar et al., 2016).

The NCu can induce dose-dependent deleterious effect not only by suppression of antibodies titre but also through inducing ROS generation andlowering antioxidants like SOD and catalase production (Kawanishi et al., 1989) leading to oxidativestress (Xin, 2015).ROSoverproduction has been showed to cause lipid and proteinper-oxidation, decline in cytochrome C oxidase activity, and mitochondrialdysfunction, followed by organ and tissue damage (Gaetke and Chow, 2003).The increased malondialdehyde levels, DNA fragmentation percent, and microscopic scoring in different organs with reduced catalase activity and antibody titre of New Castle and Avian Influenza viruses were observed in broilers that received 5 mgNCu/kg and 15 mg/kg body weight through oral gavage (Morsy et al., 2021).

Table 2. Summary of potential effect of nano copper (NCu) on poultry production performance

Reference	Species / type of poultry	NCu inclusion level (Dose)	Route of administration	Particle size	Effect on performance			
					Feed intake	Body weight	Feed conversion ratio	Egg production
Ramesh (2014)	Layers	25 and 50% of ICAR (2013) specification	Dietary	43.5 nm	Reduced	Similar	Similar	Similar
Mroczeket al. (2015)	Broilers	50 ppm	*In ovo* injection	15-70 nm	Similar	Similar	Similar	-
Scott *et al*. (2017)	Broilers	50 mg/kg *in ovo*injection + 20 mg/kg	In drinking water	2-15 nm	Similar	Improved	Improved	-
Sawoszet al.(2018)	Broilers	25, 50, 75 and 100 % of specification (7.5 ppm)	Dietary	15–75 nm	Similar	Similar	Similar	-
Kozlowski et al. (2018)	Turkeys	20, 10 and 2 ppm	Dietary	25 nm	Similar	Similar	Similar	-
Aminullah et al. (2021)	Improved breeder hens	25, 50 and 75% of ICAR (2013) specification	Dietary	50-80 nm	Reduced at 75%; Similar at 50 and 25%	Reduced at 75%; Similar at 50 and 25%	Similar	Similar
Yausheva (2021)	Broilers	2 mg/kg body weight	Intra-muscularinjection	80 nm	-	Increased	-	-
Morsyet al. (2021)	Broilers	5 and 15 mg/kg body weight	Oral gavage	28.9-45.6 nm	Similar	Reduced	Improved	-
Aminullah et al.(2022a)	Improved chicken	25, 50 and 75% of ICAR (2013) specification	Dietary	50-80 nm	Reduced at 75%; Similar at 50 and 25%	Similar	Similar	-

Table 3. Summary of potential effect of nano copper (NCu) on nutrients metabolizability in poultry

Reference	Species / type of poultry	NCu inclusion level (Dose)	Route of administration	Particle size	Effect on nutrients metabolizability				
					DM	OM	CP	EE	CF
Gonzales et al. (2009)	Piglets	50 ppm	Dietary	10-100 nm	Improved	Improved	Similar	Similar	Similar
Scott et al. (2016)	Broilers	50 mg/kg	*In ovo* injection	2-15 nm	-	-	-	Improved	-
Scott et al. (2017)	Broilers	<50mg/kg	*In ovo* injection and drinking water	2-15nm	-	-	Improved	Improved	-
Kozlowski et al. (2018)	Turkeys	20, 10 and 2 ppm	Dietary	25 nm	Similar	Similar	Similar	Similar	Similar
Aminullah et al. (2022a)	Improved chicken	75% of ICAR (2013) specification	Dietary	50-80 nm	Improved	Improved	Improved	Improved	Similar

FUTURE PROSPECTS

The application of nano technology in poultry production continues to develop and garner more attention in near future. The use of nano minerals particularly NCu in poultry nutrition can potentially improve performance of both grower and breeder type of birds including Cu tissue deposition. The effect of NCu on various parameters of poultry is graphically presented in Figure 1. Furthermore, due to the greater bioavailability and less antagonistic tendency of NCu, the supplementation level can be reduced to 75% without compromising the production performance of birds and also reducing the Cu excretionto the environment. However, the effects of NCu on different parameters in a biological system are still inconsistent. The absorption, distribution and metabolism of NCu are mainly dependent on physicochemical properties and dose of NCu. Moreover, reduced immune response and egg fertility rate were also reported due to NCu in the breeder hens' diet. More studies are required to relate the physicochemical properties of nanoparticles and their impact on various parameters in a biological system. NCu's greater antimicrobial activities against both gram-positive and gram-negative bacteria suggest that it has the potential to be an effective alternative antibiotic in poultry production in the future.Despite such advantages, a lot of research work is required to elucidate the standardization of nano-particles along with their environmental hazard, bio-safety, and quality control aspects.

Furthermore, the application of nanotechnology in animal and poultry nutrition requires special concern in risk analysis, regulatory policy and oversight. Regulatory frameworks and various approaches to ensure safety of nano products in poultry nutrition have been accredited in countries around the globe.

CONCLUSION

The studies revealed similar or improved growth performance, production rate and nutrient utilization at comparable dose or at reduced NCu levels in the diets of poultry in comparison to the conventional form of $CuSO_4$. The improved performance and nutrient metabolizability were attributed to novel properties and antimicrobial effects of NCu that improve gut health. Studies have also shown improved Cu deposition in the liver, no adverse effect on other trace minerals balance in the body as well as reduced Cu excretion to the environment when conventional form of $CuSO_4$was replaced with reduced levels of NCuin poultry diets. Dose-dependent effect of NCu on immune response indicating either improved or no effect on antibodies titre and antioxidant level were observed whereas higher levels of NCu incorporation was detrimental to immunity of birds. Further studies are envisaged to ascertain the effect of nano copper on the production performance and health status of the birds including its optimal inclusion level and toxicological studies.

Fig. 1: A Graphic illustration for effects of nano copper (NCu) on poultry

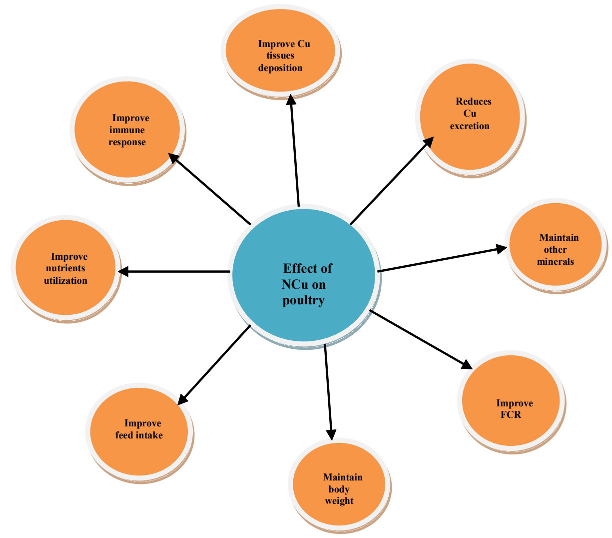

Table 4. Effect of nano copper (NCu) on mineral balance in the body tissues in poultry

Reference	Species / type of poultry	NCu inclusion level	Route of administration	Particle size	Result
Nollet et al. (2007) Gonzales et al. (2009) Sawosz et al. (2018)	Chicken	-	Dietary	10-100 nm	Reduced Cu excretion proportionally to NCu inclusion level in the diet.
Ramesh (2014)	Layers	25 and 50% of recommendation	Dietary	43.5 nm	No effect on bone mineralization, and Ca and P balance in the body.
Ognik et al.(2016b)	Broilers	5, 10 and 15 mg/L	Drinking water	1-5 nm	Reduced Ca and Zn absorption in the intestine and reduced plasma P level observed.
Mroczek et al. (2017)	Broilers	50 mg/kg	*In ovo* injection	72.3 nm	Improved bone characteristics
Aminullah et al.(2022c)	Improved Chicken	25, 50 and 75% of ICAR (2013) specification	Dietary	50-80 nm	Improved Cu deposition in the liverproportionate to NCu inclusion level
Aminullah et al. (2022c)	Improved Chicken	25, 50 and 75% of ICAR (2013) specification	Dietary	50-80 nm	Improved Cu utilization and its excretion reduced, no effect on bone mineralization and Ca, P, Zn, Fe and Mn balance in the body.

Table 5.Effect of nano copper (NCu) on immune response and antioxidant status in poultry

Reference	Species / type	NCu inclusion level	Rout of administration	Particle size	Results
Wang et al. (2011)	Broilers	50, 100, 150 mg/kg	Dietary	95 nm	Improved antibodies(IgG, IgM, IgA) level at 100 mg/kg
Ognik et al. (2017)	Broilers	54% exceeding NRC (1994)	Dietary	5 nm	Improvedantioxidant status (Interleukin-6,IgA, IgM and IgY), superoxide dismutase (SOD), catalase (CAT) and ceruloplasmin activity
Ognik et al. (2019)	Broilers	7% exceeding NRC (1994)	Dietary	5 nm	Decreased SOD in breast muscle, increased CAT and MDA in liver
Kozlowski et al. (2018)	Turkey	20, 10 and 2 ppm	Dietary	25 nm	Reduced antioxidant level – SOD, CAT, MDAat 2 ppm NCuinclusion
Ognik et al. (2019)	Broilers	7 to 25% level exceeding NRC (1994) specification	Dietary	5 nm	Suitable for liver and muscle antioxidant status - lipid peroxides, MDA, SOD,CAT and ceruloplasmin
El-kazaz et al. (2020)	Broilers	10 mg /L	Drinking water	106 nm	Increased immune organ weight Increased IgA and IgM level
Morsy et al. (2021)	Broilers	15 mg/kg BW	Oral gavage	29-46 nm	Reduced antibodies titre against ND virus.
Aminullah et al.(2022b)	Improved chicken	25, 50 and 75% of ICAR (2013)	Dietary	50-80 nm	Reduced antibodies titre against ND and IBD virus at 75%

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