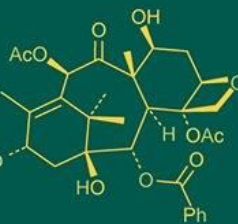
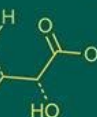
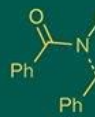
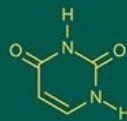
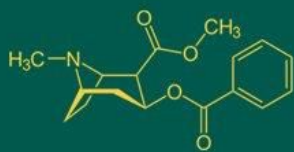


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Application of nanotechnology to enhance animal health and production

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Abstract

The advancements in nanotechnology offer exciting opportunities for its integration into medicinal and nutritional sciences, due to its minute size and larger surface area. In the realm of animal healthcare, nanotechnology is proving its importance in the diagnosis, prevention, and vaccination of various diseases. Recently, a variety of nanoparticles; such as nano-minerals, enzyme nanoparticles, probiotics, vitamins, feed additives, nano capsules, and feed antimicrobials, have been incorporated into animal feeds. The use of these nanoparticles supports a healthier gut, enhancing health and overall animal performance. This enhancement can be linked to improved nutrient absorption and utilization, which ultimately translates to increased production levels and better-quality milk, meat, and eggs. But it is essential to determine appropriate dose limits to prevent the risks associated with over-supplementation. This review emphasizes the crucial role of nanotechnology in optimizing both animal health and productivity.

Keywords: Nanotechnology, nanoparticles, nano-minerals, enzyme nanoparticles, additives, probiotics

Introduction

In Greek, 'Nano' means dwarf or very small. One billion divisions of meter are referred to as nanometers (nm). Nano replaced the factor 10^{-9} as a prefix (Grunwald, 2017) ^[37]. Involving nano-materials ranging from 1- 100 nm to design, synthesize, and apply in various devices and systems is called Nanotechnology (Pundir, 2015) ^[75]. A nano-material is an element with at least one length dimension ranging between 1 nm and 100 nm. Nanoparticles, fullerenes, carbon nanotubes, quantum dots, dendrimers, nanopores, liposomes, magnetic nanoparticles, nano-shells, etc. are common examples of nanomaterials. Among all these, nanoparticles are the most extensively studied. Nanoparticles are smaller in size and have a high surface area. Hence, the mucosal permeability is increased leading to improved absorption within the living body. At present, they are used mostly in pharmaceutical and therapeutic applications (Davda and Labhasetwar, 2002) ^[17].

History of nanotechnology

About two thousand years ago, Indian artisans and craftsmen were already applying concepts akin to nanotechnology in their creation of wootz steel and intricate paintings, even if they weren't aware of the specific structures and properties of the nanomaterials they were working with. The term "nanotechnology" was first introduced by Richard Feynman, American Nobel laureate in 1959 during his presentation, "There's Plenty of Room at the Bottom," at the annual meeting of the American Physical Society (Feynman, 1960) ^[30]. It wasn't until 1974 that But, Norio Taniguchi at 1974 defined the concept and described nanotechnology as the manipulation and arrangement of materials at the atomic or molecular level. In India, the momentum for nanotechnology research surged following the establishment of the Nano Science and Technology Initiative in 2001. This was further propelled by the launch of the Nano Mission in 2007, a five-year program initiated by the Government of India. Prof. C. N. R. Rao, the chairman of the Nano Mission, is widely regarded as the "Father of Indian Nanotechnology."

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Nanotechnology

As Booker and Boysen (2011) ^[11] depicted, nanotechnology is attributed to technological development from 1 nm to 100. It is considerably the trending division of science dealing with elements, final products with changed structures, and improved quality, at the molecular level (Usama). Undoubtedly, nanotechnology is the next industrial revolution (Schmidt, 2009) ^[90], as it holds a major role in the food production and processing chain (Otlés and Yalcin, 2008) ^[69]. Nanotechnology involves the use of nanomaterials, mostly nanoparticles. Hence, the exceptional characteristics of nanoparticles should be explored to maximize the production levels and improve the productivity of livestock and poultry.

Nanoparticles

Nanoparticles are minute particles with variable dimensions within the 1 to 100 nanometers range. Practically, these are about 1000 times tinier than the diameter of a human hair.

Applications of nanotechnology in animal health care and production

Nanotechnology is gradually taking an important place in the animal health care and production sector by offering innovative solutions that enhance the well-being of livestock and other animals. By manipulating materials at the nanoscale, researchers are developing new tools and treatments that improve disease management, boost productivity, and promote overall animal welfare. In the animal healthcare sector, nanotechnology plays a major role in treating various animal diseases, including diagnosis, prevention and vaccination (Busra Dumlu, 2024) ^[13].

Diagnosis of diseases

Nanotechnology is proving to be a game-changer in the development of diagnostic tools for diseases, offering quick and accurate detection at a low cost. The outbreak of avian influenza (H5N1) in 2006 led to significant economic repercussions (El Sabry *et al.*, 2012) ^[22]. Since then, innovative tools have emerged that leverage the exceptional properties of nano-materials to identify pathogens and forecast outbreaks like bird flu. For instance, Emami and his team utilized nanotechnology to detect avian flu antibodies in bird serum. They incorporated gold nanoparticles as a protective layer over a specific membrane in their Western blot technique, significantly enhancing both the accuracy and sensitivity of the test. In another advancement, researchers from the University of Guelph in Canada developed a nano-detector to identify bird flu outbreaks in poultry farms (Researchers Guelph University, 2015). This innovative detector employs gold nanoparticles and quantum dots, requiring only a minimal blood sample. It is not only more affordable and quicker but also boasts improved accuracy. Furthermore, to detect *Mycobacterium bovis*, which causes bovine tuberculosis in cattle, Peled and his team created sensors integrated into a nano artificial nose known as NA-NOSE (Peled *et al.*, 2012) ^[73]. This remarkable device can analyze the breath of cattle to identify those infected with *Mycobacterium bovis*.

Veterinary Medicines and Drug Delivery

According to the definition given by the Scientific Committees on Consumer Safety, the application of nanotechnology to diagnose, treat, and prevent the

occurrence of diseases is called nanomedicine (The Scientific Committees, 2013). The basis of nano-medicine is the 'smart drug delivery system' to the target tissue as per the requirement (Scott, 2005) ^[91]. The drugs are encapsulated by nano-particles which are biodegradable to get protected from digestive fluids, as well as the absorption will be better (Des Rieux *et al.*, 2006; Cai *et al.*, 2010; Simon *et al.*, 2009) ^[19, 15, 99]. By the administration of oral nano-medicine, the absorption pattern is uniform, and the chances of local irritation can be minimized (Galindo-Rodriguez *et al.*, 2005; Feng *et al.*, 2009) ^[33, 27].

Vaccines

The nanoparticles function in two ways to improve the efficacy of the vaccine; delivery carriers and new adjuvants. The enhanced effectiveness of nano-vaccines is conspicuous from different investigations. Among the nanoparticles, the biodegradable ones are more advantageous revealing superior antigen stability, higher immunogenicity, and targeted vaccine delivery (Sekhon, 2014) ^[92]. Examples of some nanoparticles being used to elicit cell-mediated immunity and antibodies are gold nanoparticles, silica, latex, and polystyrene. (Pavelic *et al.*, 2001) ^[72]. For nasal vaccines, encapsulation with bioactive nanoparticles holds good result.

Treatment

The cutting-edge strategy of nanomedicine and research is the judicious delivery and targeting of pharmaceuticals, medicines, and vaccines (Desai *et al.*, 1997) ^[20]. Encapsulation with nanoparticles improves the solubility, and diffusion of the pharmaceuticals or medicines and can carry them to the target site. Some commonly used nanoparticles for the treatment of veterinary diseases are; carbon nanotubes, dendrimers, nano-shells, nanopores, liposomes, magnetic nanoparticles, polymeric nanoparticles etc.

Antimicrobials

To combat with the harsh effects of antimicrobial resistance, nanoparticles offer a viable alternative to antibiotics. Metal nanoparticles possessing positive charges can result in the lysis of negatively charged bacterial membranes (Gahlawat *et al.*, 2016; Xu *et al.*, 2015) ^[34]. The antimicrobial effects of silver nanoparticles were studied by Kim and his coworkers (Kim *et al.*, 2007). They found the inhibitory action of silver nanoparticles on the growth of *Escherichia coli* causing haemorrhagic enteritis and yeast causing bovine mastitis. Dobrzanski and his coworkers investigated that, application of nano silver decreased the count of *Escherichia coli* and some other mesophilic bacteria in litter (Dobrzanski *et al.*, 2010) ^[21]. Nano-copper is also frequently supplemented in feeds as a mineral supplement and antimicrobial agent. The antimicrobial activity of chitosan, a positively charged biopolymer was well documented by Qi *et al.* (2004) ^[76].

Reproductive Aids

Nanoparticles can be used as reproductive aids as, some of the nanoparticles help in improving fertility and protecting the spermatozoa (Hill and Li, 2017) ^[42]. In artificial insemination, nanotechnology can be integrated in various processes like bioimaging of gametes, purification of sperms, and protectants. Purification of sperm is done to remove the damaged spermatozoa, which can be recognized

by the specific antibodies combined with nanoparticles (Odhiambo *et al.*, 2014; Petruska *et al.*, 2014; Hill and Li, 2017) [67, 74, 42]. To visualize the movements of mammalian spermatozoa and oocytes at the molecular level during the fertilization process, some fluorescent inorganic nanoparticles and quantum dots are preferably used (Feugang *et al.*, 2015; Hasuwa *et al.*, 2010) [29, 40].

Molecular Biology Agents

The major bionanomaterials contributing to the different developmental processes are DNA, RNA, and peptides. Transfer of genetic materials can be mediated through interactions between nucleic acids and the nanoparticles (Agarwal *et al.*, 2007; Barkalina *et al.*, 2014) [4, 10]. By the use of gold nanoparticles, magnetic nanoparticles, silica nanoparticles, quantum dots, fullerenes, carbon nanotubes, cationic lipids, and cationic polymers; *in vitro* and *in vivo* delivery of genes can be achieved (Katragadda *et al.*, 2010) [49]. Apart from this, for effective diagnosis of the targeted organs; quantum dots, nano-shells, and carbon nanotubes can be used (Neeraj Dilbaghi *et al.*, 2013) [66].

Nanotechnology in animal nutrition

Recently, different types of nanoparticles like nano-minerals, enzyme nanoparticles, probiotics, vitamins, and feed additives have been used in the feeds (Zaheer, 2021; Fesseha *et al.*, 2020; Marappan *et al.*, 2017; Pundir, 2015) [75, 28, 61, 75].

Nano-minerals

Minerals, especially trace minerals like selenium, chromium, copper, silver, zinc, iron, and manganese are available in nano form. Nano minerals can be added to the primary feed ingredients (Schmidt, 2009; Gelaye, 2024) [90, 35] with a lower dose rate due to the tiny particle size and great surface area. This lower dose reduces the ill effects of some essential toxic minerals like selenium. Small size is also attributed to better bioavailability, absorption rates, and digestion by easily passing the gastrointestinal mucosa layer (Bunglavan *et al.*, 2014; Jafari & McClements, 2017) [12, 47]. Various research works have been conducted to find the effects of the nano minerals in livestock production.

Nano selenium exerts antioxidant properties and effects on the reproductive system even in a lower dose (Sadeghian *et al.*, 2012) [83]. In broiler chicken, body weight gain, feed efficiency ratio, and Se retention are higher in the nano selenium-supplemented group than in the sodium selenite-supplemented group (Hu *et al.*, 2012) [43]. In layer chickens, supplementation of nano-Se improved different biochemical and haematological parameters leading to better health (Mohapatra *et al.*, 2014) [64] and enhanced fertility, sperm quality, and egg production leading to increased production (Nabi *et al.*, 2020) [65]. Ultimately, compared to the inorganic form of selenium, nano form of selenium was found to be more effective for selenium bioavailability in different tissues and high growth rate in the animals (Hu *et al.*, 2012) [43].

Chromium has a significant impact on glucose tolerance by enhancing the action of insulin. In a study involving finishing pigs, supplementation with nano chromium led to increased levels of immunoglobulins, specifically Ig M and Ig G, in the plasma. Additionally, it resulted in elevated serum insulin-like growth factor I while decreasing serum insulin and cortisol levels (Wang *et al.*, 2012) [96].

Furthermore, nano-Cr positively influenced the characteristics and quality of pork, improved the muscle mass, and increased the bioavailability of chromium in different organs (Wang and Xu).

Research indicates that incorporating nano silver as a feed additive can have a beneficial effect on the bacterial populations within the digestive tracts of poultry. In a study where feed was supplemented with nano silver at different concentrations, there was a notable decrease in the weight of lymphoid organs, highlighting a dose-dependent response (Ahmadi, 2012) [6]. Additionally, Andi *et al.* (2015) [8] found that broilers receiving nano silver nanoparticles experienced improvements in the intake rate and efficiency of feed.

In a study involving piglets, it was found that supplementing their diet with nano copper (Cu) increased the concentration of immunoglobulins as well as enhanced the activity of the enzyme superoxide dismutase (SOD) (Gonzales-Eguia *et al.*, 2009) [36]. Another research focused on nano zinc demonstrated that when broiler birds were provided with a diet containing 0.06 ppm of it, their immune status and nutrient absorption improved significantly compared to those receiving inorganic zinc (Sahoo *et al.*, 2014) [84]. Moreover, nano zinc supplementation not only bolstered the immune status of broiler birds but also played a role in inhibiting the growth of mycotoxic fungi and reducing mycotoxin presence, ultimately minimizing feed treatment risks.

Enzyme Nanoparticles

Enzyme nanoparticles are enzymes prepared artificially and made up of nanomaterials. There are two major groups of enzyme nanoparticles are; oxidoreductases (peroxidase, oxidase, catalase, superoxide dismutase) and hydrolases (protease, nuclease, phosphatase) (Wu *et al.*, 2012) [14]. Compared to the commercially available natural enzymes these are biologically more compatible, thermally stable, more active, and more sensitive. These improved characteristic features are due to the smaller size and enhanced surface area (Eivazzadeh-Keihan *et al.*, 2022) [23]. Some other important characteristics of enzyme nanoparticles are wider temperature tolerance capacity and better digestion of cellulose (Gu, Liu).

Probiotics

Nanotechnology has a great role in the designing of new probiotics with greater nutrient bioavailability and food quality. It is due to the fact that, nanomaterials can enhance the bioavailability by a higher level of adhesion to mucosal membrane and better interactions with different enzymes (Reque & Brandelli, 2021) [81]. One important example is Nano Copper, which is generally used as a probiotic. It also helps in improving the nutritional value of the feed. Some other examples of nano probiotics are; zinc nanoparticles and curcumin nanoparticles. These nanoparticles when supplemented to the broilers along with *Bacillus licheniformis* caused increase in body in weight and higher meat quality (Abd El-Hack *et al.*, 2021) [1].

Feed Additives

Feed additives are ingredients or combinations of ingredients added to animal feed to meet specific goals such as promoting health, enhancing production levels, and improving feed quality. By utilizing nanotechnology, these additives are created with smaller particle sizes, which

enhances their surface area. This increase in surface area boosts their digestibility, absorption, and metabolic efficacy (Hatab *et al.*, 2022; Hussain *et al.*, 2023) [41, 45]. As a result, these improvements can positively influence growth, development, and immune function (Sharif *et al.*, 2021) [94]. Examples of nano feed additives include vitamins, antioxidants, colouring agents, and flavouring agents. Notably, coiled nano chelates of 50 nm are effective in delivering nutrients like vitamins, lycopene, and omega fatty acids without changing the feed's colour or taste (FSAI, 2008) [32]. In studies with rabbits and other monogastric animals that were fed diets containing nano emulsified essential oils, researchers observed increases in final live weight and carcass weight, as well as improvements in meat protein content and a reduction in fat content, alongside higher levels of monounsaturated and polyunsaturated fatty acids (Abdelhadi *et al.*, 2022) [1].

Nanocapsules (Encapsulation)

To enhance the bioavailability of active ingredients such as minerals, vitamins, essential oils, antioxidants, enzymes, and coenzymes while preventing oxidation, nanoparticles can be employed for encapsulation (El Amin, 2006). The products created through this method are known as Nano capsules (Shah *et al.*, 2016; Pateiro *et al.*, 2021; Tolve *et al.*) [93, 70]. Additionally, natural food or feed ingredients, like protein micelles, can also be encapsulated using nanoparticles (Singh, 2012) [60]. This encapsulation technique effectively masks any unpleasant colour and odour of the products and protects against negative environmental impacts.

Feed Antimicrobials

Nanoparticles could offer a promising solution to the increasing risks of antimicrobial resistance in microorganisms. Specifically, metal nanoparticles like Nano Copper and Nano Silver have shown potential as effective antimicrobial agents.

Feed Processing

In feed processing, the use of nano sized or nano-encapsulated food additives, especially, fat-soluble additives, help in enhancing the dispersibility, improving the taste, maintaining the storage of food and reducing the use of excess fat (Anju Manuja *et al.*, 2012) [60]. Innovative nanomaterials are being developed to better protect feed (Ross *et al.*, 2004) [95].

Effects of inclusion nanoparticles on animal feed

The addition of nanoparticles contributes to a healthier gut, which in turn enhances the overall performance of the animals. This improved performance is attributed to better absorption and utilization of nutrients, leading to increased production levels and higher quality milk, meat, and eggs, as well as stronger immune responses within the gastrointestinal tract.

Better Growth Performance and Nutrient Absorption

Nanoparticles are crucial for boosting nutrient absorption by reducing the adverse effects of bivalent cations, especially with smaller minerals. This benefit is particularly noticeable in livestock and poultry, as it enhances their nutrition and optimizes the usage of feed and supplements (Marappan *et al.*, 2017) [61]. For example, research indicates that nano ZnO

contributes to growth and improves feed efficiency in both piglets (Yang *et al.*, 2018) [53] and poultry (Mishra *et al.*, 2014; Lina *et al.*, 2009) [65, 55]. Furthermore, a study by Zhou and Wang (2011) showed that incorporating 0.30 mg/kg of nano-Se in diets significantly improved the growth rate and feed efficiency in chickens, while also boosting selenium levels in their tissues and enhancing meat quality. In another findings, Shi *et al.* (2011) [96] discovered that selenium supplementation not only enhanced the growth but also improved the selenium bioavailability in blood and other tissues of growing male goats.

Improve the Egg Production and Egg quality

Radwan *et al.* (2015) [78] studied the effects of incorporating nano-selenium and sodium selenite into the diets of laying hens. Their research revealed that hens supplemented with nano-selenium experienced improvements in both egg production and feed conversion ratios. Similarly, Salah-Eldin *et al.* (2015) [78] examined the effects of selenium-methionine nanocomposites, finding a noteworthy increase in egg mass ($p < 0.05$) with elevated selenium levels. Additional studies by Tsai *et al.*, Olgun and Yildiz (2017) [68], and Abedini *et al.* (2018) [3] showed that zinc oxide nanoparticles had a beneficial effect on zinc retention, along with enhancement in the production and quality of eggs, improved immunity, and serum aging parameters. Furthermore, research conducted by Sirirat *et al.* and Sathyabama and Jagadeeswaran (2016) [89] found that chromium picolinate nanoparticles notably improved egg quality. Amiri, Andi, and Shahamat (2015) [8] also noted that the addition of nano-chromium to the feed for Japanese quail resulted in better egg quality parameters.

Improve the Composition and Quality of Meat

Recent studies have shown that supplementing with Nano selenium leads to significant improvements in several areas for broilers, including weight gain, selenium status, antioxidant activity, feed conversion rates, and meat quality (Ahmadi *et al.*, 2018; Cai *et al.*, 2012; Li *et al.*, 2018; Zhou and Wang, 2011; Liu *et al.*, 2017) [5, 14]. Likewise, research involving piglets indicates that the addition of nano copper (Gonzales-Eguia *et al.*, 2009) [36] and nano chromium (Wang) also enhances carcass characteristics, pork quality, muscle mass, and overall growth performance.

Improve the Milk Production and Quality

Nanotechnology has the potential to enhance milk safety for human consumption. For instance, research by Sung *et al.* introduced nanocomposites consisting of anti-*Staphylococcus aureus* antibodies, gold nanoparticles, and magnetic nanoparticles, enabling a rapid 40-minute colorimetric test for detecting *S. aureus* in milk. Similarly, Wang *et al.* (2011) [96] showcased a method for identifying toxins in milk by utilizing polyclonal antibodies alongside gold nanoparticles. In another study, Lee *et al.* (2015) [52] incorporated nano powdered Oyster shells into milk to boost calcium levels from 100 to 120 mg/ ml. Additionally, Rajendran (2013) [79] found that supplementing with nano ZnO led to increased milk production.

Effects on Rumen Fermentation Pattern

Nano selenium supplementation in the diet of sheep can modify the pattern of fermentation in the rumen, by increasing the production of propionate, which in turn

reduce the acetate: propionate ratio (Shi *et al.*, 2011)^[96]. In a study by El-Nile *et al.* (2023)^[25], natural zeolite in nano form, was introduced as a feed additive, which ultimately resulted in improved rumen fermentation pattern, blood metabolites, milk production and feed intake in dairy goats. The findings showed a boost in milk production and short-chain fatty acids. Furthermore, incorporating nano selenium into the basic diet has been observed to enhance rumen fermentation and feed efficiency, likely by promoting the activity of rumen microbes, digestive microorganisms, or enzymes (Shi *et al.*, 2011)^[96].

Improve the immune responses

Researches conducted by Sahoo *et al.* (2014a; 2014b)^[84, 85] found that adding nano zinc to the diet of broiler birds at a concentration of 0.06 ppm considerably enhanced the immune response compared to the conventional dosage of 15 ppm, whether it was organic or inorganic. Additionally, Saki and Salary (2014)^[86] revealed that chicks hatched from eggs treated with nano-silver exhibited significantly stronger humoral immunity in their laying hens.

Risks and challenges

The health risks associated with nanoparticles remain largely uncharted, as our understanding of them is still in its infancy. Due to their unique characteristics, nanomaterials may cause unforeseen environmental and health risks (Srinivas *et al.*, 2010)^[95]. It's crucial to establish appropriate dosage limits to prevent the dangers of over-supplementation. Issues concerning safety thresholds and toxicity are significant barriers to the commercial use of nanoparticles (Radha *et al.*, 2014)^[77]. Additionally, certain insoluble nanoparticles have the potential to penetrate the blood-brain barrier (Singh, 2012)^[60]. Furthermore, enzyme nanoparticles might reduce feed efficiency by degrading unintended components of feeds (Islam *et al.*, 2023)^[46]. The elevated cost of nano- feed additives also present a challenge that cannot be overlooked (Dumlu, 2024)^[13].

Conclusion

Nanotechnology offers promising possibilities for enhancing livestock growth and production. Although this technology is still evolving and being explored for various applications, research in this arena remains somewhat limited. It's essential to conduct more in-depth studies to better grasp how nano feed additives impact various animal species, their particular uses, economic benefits and implications for sustainability.

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