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Review Article

Nanoparticles in Equine Nutrition: Mechanism of Action and Application as Feed Additives

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ABSTRACT

Several concerns exist regarding horse rearing such as environmental pollution, antibiotics resistance, digestive disorders, mycotoxins contamination of animal feed, gut health management, and improvement of feed efficiency. Nanoparticles have the potential to address these issues and thus could be used as feed additive. Citrate reduces and stabilizes gold nanoparticles, alongside biosynthesized silver nanoparticles have the potential to prolong and improve digestive enzyme activity, which would enhance starch digestibility in the stomach. Zinc oxide and selenium nanoparticles could be used to improve feed digestibility and volatile fatty acids production. Magnesium oxide, silver, and copper nanoparticles exhibit strong antimicrobial activity against gram-positive and gram-negative microbes and weaken the biofilm formation of the microbial community. Calcium, zinc, and silver nanoparticles could be used to prevent periodontal disease in horses. In addition, silver nanoparticles may be applied as antifasciolitics and potentially against other gastrointestinal parasites. Environmental concern of equines could be addressed by using cerium oxide, silver, and cobalt nanoparticles to reduce methane emission and zinc oxide could help to reduce fecal mineral output. Fullerol $C_{60}[OH]_{24}$, a honey-derived silver nanoparticle and zinc oxide nanoparticles exhibit attractive antibacterial properties because of increased specific surface area as the reduced particle enhance unit surface reactivity. Gut health management of equines could be solved with nanoparticles because of the ability of ferrous oxide and copper nanoparticles to improve microbial growth, whereas zinc oxide improves villus height, crypt depth, and villous surface area. It is required to explore in depth the beneficial effects of these nanoparticles as a novel area in the equine industry's both in vitro and in vivo before recommendation to equine owners.

1. Introduction

Throughout history, human activities and development have constantly undergone changes and transformation. One of the new techniques in the phase of development is nanotechnology. Humans usually transfer applicable technology/technique in other fields to animal production. Hence, the applications of nanoparticles in agriculture, especially as feed additives, health enhancers, and antimicrobial in animals, are increasing. Nanotechnology is the technique of using organic and inorganic matter, in minute-sized quantity usually between 1 to 100 nm to incorporate nanosized materials for medical, antimicrobial, drug delivery, electronics, cosmetic activity, food packaging, and encapsulation with enhanced efficiency. Nanoparticles may be defined as a small object that behaves as a whole unit for its transport and owing to its high level of bioavailability and biodegradability [1].

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Several challenges occur in equine rearing, which are being addressed such as environmental pollution, antibiotics resistance, digestive disorders, fungal metabolites found as toxic contaminants of feed, gut health management, and improvement of feed efficiency. To address these challenges, nanoparticles are used as alternative antimicrobial agents to overcome the use of antibiotics, which had spread around the world at an alarming rate against pathogenic bacteria, as feed additive, against mycotoxins, and as biocidal agents [2]. Manipulations to improve equine productivity or efficiency have been carried out through dietary supplements such as yeast, probiotics, fibrolytic enzymes, and plant extract. Nanoparticles are new feed additive that may be used in modern animal nutrition. Nano feed additives may be used in animal feed and health treatments [3]. The promising results in other livestock such as poultry, pigs, and sheep could be translated to equine nutrition. Organic nanoparticles are likely to be used to enhance the nutrient value of feed systems because of bioavailability [4]. These nanominerals showed their significant beneficial effects even at doses lower than the conventional mineral sources [5]. Nano feed additives could help in improving feed efficiency, reducing feed cost, and could be used for fat protection to reduce intestinal fermentation disruption [6].

Nanoparticles have unique features such as small size, high surface area, surface charge, high catalytic efficiency, stronger adsorbing ability [7], and exhibit higher absorption efficiencies. The use of nanotechnology such as nanoencapsulation and nanoparticles in animal nutrition, to ensure minute addition of materials in smaller particulate, and to perform the same function as bulk-sized counterpart may be referred to as “nano nutrition/nano additives”. Metal and phytobased synthesized nanoparticles are used in animal nutrition research such as silver, gold, calcium, iron, selenium, silicon, titanium, and zinc which are used in various fields of nanotechnology research.

The recent development in these fields of nanoscience and nanotechnology could be transferred to equine production system and/or nutrition with applications such as feed additives to facilitate health and improve productivity, mineral bioavailability, removal or inactivation of toxins in animal feeds. In addition, they have enormous potential to mitigate environmental problems. Nanoparticles are expected to have an advantage of bioavailability, small dose rate, and stable interaction with other components [8]. It is important to note that there are extremely limited articles on the use of nanoparticles in equines. Hence, this review is meant to encourage equine researchers to expand the knowledge on the potential of nanomaterials.

2. Type of Nanoparticles Used in Animal Research

Common nanoparticles used in animal production and feed additives are polymeric, liposome, dendrimer, micellar nanoparticles, and ceramic nanoparticles. Carbon-based nanoparticles such as fullerene and carbon nanoparticles which are organic and metallic inorganic nanoparticles have also been used. Metallic nanoparticles are commonly used nanomaterials in livestock production such as gold, silver, cobalt, copper, chromium, magnesium oxide, ferrous oxide, zinc oxide, titanium oxide, and selenium. Nanoparticles are also synthesized from plants such as *Azadirachta indica*, *Camellia sinensis*, and *Aloe vera,* which are known as green nanoparticles. Nanoparticles have veterinary application, used for destruction of cancer cells, drug delivery system, antimicrobial agents [9,10], application in waste treatment, and to reduce bleeding (Hangge). Toxicity of nanoparticles has been observed because of their accumulation in the liver, and lung tissue of sheep, rats, and fishes, as well as the brain in laboratory animals [10]. The properties, formation, and sizes of these nanoparticles [2,11], syntheses such as activated carbon from biowaste, honey-mediated nanoparticles [12,13], application [5–7,14,15], and toxicity [16–18] have been thoroughly reviewed.

3. Mechanism of Action

A common way of action of nanoparticles is to trigger oxidative damage through inducing reactive oxidative species, cell membrane disruption, and the inhibition of cell division and cell death [19–22]. The excessive formation of reactive oxygen species including hydrogen peroxide leads to oxidative stress and subsequent cell damage [23]. Others include depletion of intracellular ATP production and disruption of DNA replication [24].

Specifically, some nanoparticles have been biofunctionalized to serve as an alternative to materials such as mannose, which create attraction for bacteria adhesion through mannose receptor sites [25,26]. Furthermore, the antimicrobial activity of carbon nanoparticles may be attributed to the electrostatic repulsion between gram-negative microorganisms and carbon surface whereby microbes adhere to carbon particles through strong van der Waals forces and modification of positive charge on carbon surface in gram-positive bacteria [27]. Carbon nanoparticles penetrate into the cell and prevent bacteria cell division and cell death, which results in cell lysis [22,28].

Silver nanoparticles inactivate enzymes, change expression of protein, and damage the respiratory chain, which destroys biomacromolecules [29]. Nanoparticles also act by disrupting bacterial cell membrane or bursting bacteria membrane through its hydrophobic chains of certain lengths [25,30]. Polymeric nanoparticles kill microbes on contact with bacterial cells because of the strong interaction of their cationic surfaces with the bacterial cells [31]. Furthermore, nanopiercing results by the action of nanoparticles prickly structure such as zinc-doped copper oxide prickly nanoparticles, which have high killing efficiency due to bacteria cell wall disruption [32]. Nanoparticles disrupt microbial membrane and hinder biofilm formation, which could aid antimicrobial activity. Biofilms are specifically microbial aggregates that rely on a solid surface and extracellular products, such as extracellular polymeric substances [33]. Thus, biofilms are a serious health threat [34,35], and thus hindering biofilm formation could increase susceptibility of pathogenic microbes. However, acetate kinase and coenzyme F420 are important enzymes in the methanation processes. The acetate in this process is converted to methane; hence, nanoparticles inhibit methane emission by reducing the population of Achaea and the suppressing acetate kinase and coenzyme F420 [36]. Owing to the different engineering techniques and types of nanoparticles, nano-based feed additives can facilitate a wide range of functions as shown in Fig. 1.

4. Potential Application of Nanoparticles in Equines

4.1. Nanoparticles and Feed Digestibility

Digestive disorders in equines have been linked to feeding high-starch grain diets [37]. This is because undigested or poorly digested starch in the stomach of equines enters the cecum-colon chamber where anaerobic fermentation takes place, which disrupts the normal microbial balance/activity due to depression of hindgut environmental pH. Hence, if digestion of soluble carbohydrates such as starch/grains in the stomach is enhanced, a lower flow of starch to the hindgut occurs. Yeast is useful in improving starch digestibility and consequently reducing volatile fatty acids production [37]. Nanoparticles are also capable of doing similar things. Nanoparticles could also be used in equine diets to improve starch digestibility. The activity of digestive enzymes such as

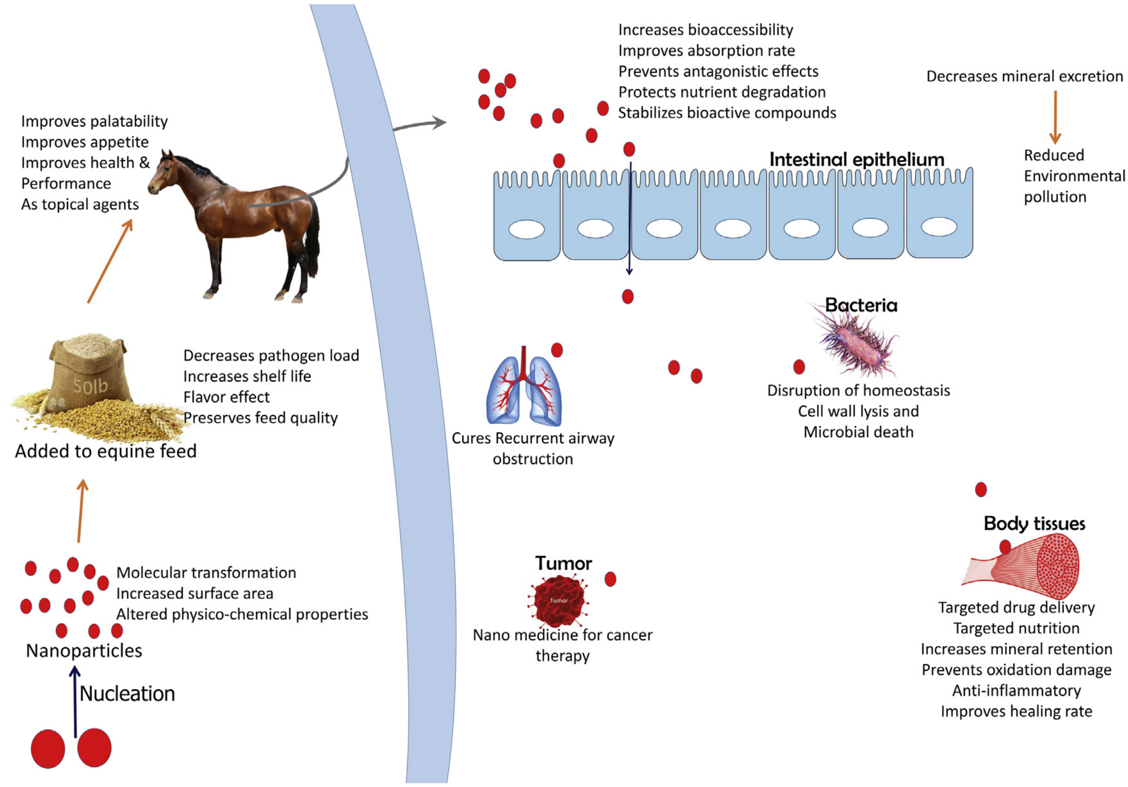

Fig. 1. Mechanism of action of various nanotechnology-based feed additives in improving the equine health and well-being.

protease, amylase, and lipase could be used as indicator of potential feed utilization [38] and to a certain extent, digestive capacity based on feed offered.

The study of Saware et al [39] showed an increased enzymatic activity of α-amylase in the presence of citrate-reduced gold and biosynthesized silver compared with the control (amylase) without nanoparticle (free enzyme). The α-amylase activity increased by more than 1.5-fold in the presence of gold and silver nanoparticles with more activity with gold nanoparticle present. Further studies showed that the enzymatic activity increased with increasing starch solution (0, 0.25, 5, 10, 20, 30, 40, and 50 mg/mL). This demonstrates the nanocatalyst activity of the nanoparticles due to their increased breakdown of starch to reducing sugar. Thus, the use of nanoparticles could have a catalytic activity on starch intake, which would reduce starch granules flowing to the equine hindgut; hence, reducing microbial dysbiosis. The reason for this increased activity compared with soluble (free) enzyme is because the immobilized enzyme that made the solid nanoparticles overcame the common collision frequency that occurs between free enzyme and the molecules of the substrate [39]. In addition, efficiency of enzyme on solid support increases when compared with its free form [40]. Furthermore, enzymes are attached to the nanoparticle after the degradation of starch from the composite and not to the reducing sugars, although the enzymatic activity was retained [41]. This may also be applied to fibrolytic enzymes in equine diets to increase fiber digestibility. The efficiency of enzymes may reduce the quantity of fibrolytic enzymes used in equine diets.

Similarly, citrate-stabilized gold nanoparticles with an average size of 11 nm increased α-amylase activity with increasing starch concentration [42]. It was observed that 0.175, 0.35, 0.70, 1.40 μg/mL of α-amylase resulted in about 9-, 6-, and 5.5-fold increase in α-amylase concentration when probed with starch in the presence of gold nanoparticles. The decrease in enzymatic activity with increasing α-amylase is due to the attachment of the enzyme to the nanoparticles and the subsequent agglomeration, which reach the active sites of the enzyme inaccessible to starch molecules, which decrease the rate of reaction [42].

Another study by Muralisankar et al. [43] found that supplementing zinc nanoparticles at 60 parts per million (ppm) level showed improved activities of digestive enzymes (protease, amylase, and lipase) in aquaculture. In fact, enzymatic activity such as in trypsin and peroxidase may be prolonged for weeks instead of few hours when attached to nanoparticles such as magnetic Fe [42], which would help to improve metabolic activity, and digestion of feed consumed by equines. Volume of gas produced in vitro after feed incubation may be used as energy fed value and feed digestibility because of the estimation of short chain fatty acids and organic matter digestibility. Chanzanagh et al [44] showed that in vitro digestion of plant protein using 30 ppm of nano-ZnO resulted in 3.68% more gas production for soybean meal, 11.63% for rapeseed meal, 14.71% for cottonseed meal, in 24 hours fermentation period. Consequently, short-chain fatty acid production increased by 3.71% for soybean meal, 11.70% for rapeseed meal, and 15.0% increase for cottonseed meal. The increase in in vitro gas production and short chain fatty acid may be attributed to improved microbial activity, which was aided by the nanoparticles inclusion. Application of Zn improves fermentation and increases the energy value of the diet [45]. Supplementation of 4 g/kg dry matter (DM) nanoselenium (supply 4 mg selenium) resulted in 12.7, 11.3, 9.3, 19.5, and 16.6% increase in DM, organic matter, crude protein, acid detergent fiber, and neutral detergent fiber (NDF) digestibility, respectively [46]. Nanoselenium supplementation resulted in 17.3% and 5.7% increase in propionic acid and total volatile fatty acids, respectively, and 24.43% decrease in ammonia nitrogen when fed to sheep. Furthermore, in situ DM and NDF digestibility of *Leymus chinensis* was improved by 81.9% and 103.6%, respectively, compared to control diet. The increased digestion may be due to large specific surface areas which improve adequate nutrient binding and biological interactions [47]. Furthermore, there is an enhancement of adsorption capacity due to the high amounts of Gibb's free energy in nanoparticles [48].

4.2. Microbial Activities and Health Benefit of Nanoparticles

Animal body coexists with a myriad of microbes, which could be beneficial or pathogenic. One of the objectives of using nanoparticles in animal feed is to reduce the population of pathogenic microbes and stimulate the growth of beneficial microbes [49]. A great number of metal and nonmetal nanoparticles such as copper, gold, silver, silica, zinc oxide, carbon-based nanomaterials possess biocidal properties [50]. High dosage of heavy metals such as Zn and Cu is an important contributor for maintaining an adequate antimicrobial resistance [51]. Antibiotics therapy is applied to horses for preventing and treating bacterial infections [52,53], which help to reduce the rate of infection, death losses, and reduce overall performance [54,55]. However, clinically relevant antibiotic potentially causes diseases such as diarrhea or colitis in equines [54,56]. Nanoparticles are a promising alternative to antibiotics with the additional advantage of promoting growth due to their high bioavailability [14].

Nanostructured MgO is low cost, easy to manipulate, and show intrinsic biocompatibility [57]. This nanoparticle presents antimicrobial activity at 0.7, 1.0, and 1.4 mg/mL against *Escherichia coli* and *Pseudomonas aeruginosa,* by damaging the cell wall, cell membrane, and the destruction of formed biofilms [58–60]. Nanoparticles may be used as prebiotics in animal feeding [61]. Similarly, an increase in the population of *Lactobacillus* and *Bifidobacterium* in cecal digesta and decrease in coliforms population occurs when copper-loaded chitosan nanoparticles are used [62]. Supplementation with graded level of 0, 25, 50, and 75 mg/kg copper nanoparticles increases growth of total bacteria, which was in a range of 45.97%–105% compared with the control [63]. This is an indication of the probiotic potentials of nanoparticles. Furthermore, pathogenic *E. coli* and *Clostridium* spp. were reduced by about 1.6- to 2.7-fold and 1.37- to 2.86-fold, respectively, compared with control with nanoparticle supplementation.

Dental diseases and digestive disorders are health concerns in equines [64]. Horses suffer from a variety of oral/dental diseases that have polymicrobial infectious etiologies, such as caries, endodontic or periapical infections, and periodontal disease [65]. Periodontitis is a chronic inflammatory disease characterized by destruction of the tooth-supporting structures [cementum, periodontal ligament, and bone]; if untreated tooth loss can occur [53]. The use of local and systemic antibiotics and topical application of antiseptics are main ways to treat this disease which has shown mixed results [55,64].

Treatment of periodontitis bacteria with calcium, zinc, and silver nanoparticles increased biofilm formation but this biofilm was susceptible to detachment [55]. Biofilm formation is the means by which microbes reduce the impact of antibiotics [66] by hiding themselves within the polymeric matrix [67]. Specifically, the number of bacteria [CFU/biofilm] for *Veillonella parvula* NCTC 11810 and *Porphyromonas gingivalis* ATCC33277 were lower than the control when calcium and silver nanoparticles were used. Similarly, *Streptococcus oralis* CECT 907T bacterial growth was lower than the control when treated with Ca, Zn, and Ag. This is attributed to the antiseptic potential of the metal cation [53]. The initial increase in biofilm formation may be attributed to the surface charges of nanoparticles, which attract bacteria membranes. Furthermore, the cation on the nanoparticles attracts the anion charge of the bacteria membrane of −20 mV at neutral pH [68]. Silver nanoparticles possess antimicrobial activity among the bacteria isolated from horse dung [56]. Hence, inclusion of such nanoparticles in equine water, or giving them to horses orally could help to alleviate or reduce periodontal pathogens. However, *Actinomyces naeslundii* ATCC 19039, *Fusobacterium nucleatum* DMSZ 20482, and *Aggregatibacter actinomycetemcomitans* DSMZ 8324 grew more in the nanoparticle-treated group than in the control. Still, it was observed that despite their increased growth, the 72 hour biofilm formed was weakened. This showed that even with high growth of pathogenic bacteria, nanoparticles acted as a weak link among the biofilm, which aided in their breakdown and caused cell death [53].

Fascioliasis is caused by a liver fluke belonging to the genus *Fasciola*. A large number of farm animals, such as sheep, goats, cattle, buffalo, horses, donkeys, camels, and rabbits, present a prevalence rate up to 90% in some areas [69]. Silver nanoparticles of 4.6 nm produced by *Trichoderma harzianum* were tested in vitro on its ability to inhibit egg hatching compared with conventional drug triclabendazole. It was observed that nanoparticles inhibited egg hatching of *Fasciola* by 28.71% compared with a conventional drug [70]. This action of the nanoparticle may be due to perforation of the cellular membrane, which looks like cell wall pits, which could cause cytoplasmic leakage and probably inactivated respiratory chain [32] and result in the death of such egg [59].

4.3. Physiology

Supplementation of copper nanoparticles at graded level of 0, 25, 50, and 75 mg/kg copper nanoparticles level improved the growth rate and plasma superoxide dismutase (SOD) (u/mL) with 50 and 75 mg/kg having the highest response, respectively [63]. Superoxide dismutase helps to prevent inflammations derived from sports injuries, which is indubitably important for equines. Similarly, pH and ammonia in copper nanoparticles-supplemented rabbits were lower than the control. The low pH was in a range of 6.17–6.57. Low pH may sometime mean an indication of rapid digestion of soluble substrate, which drops pH. The pH did not pose any risk of acidosis. The low ammonia nitrogen may be attributed to the improvement in the utilization by fermenting microbes. Similarly, inclusion of 60 mg nano-ZnO/kg diet and 30 mg Zinc Oxide nanoparticle (nZnO)/kg in the diet of rabbits increased serum SOD threefold and twofold, respectively, compared with normal 60 mg nano-ZnO/kg supplementation [4].

So far, the major source of Zn for animal feed supplementation has been its inorganic salts, such as zinc sulfate, zinc oxide, and zinc chloride [71]. However, the use of this mineral as a growth promoter is one of crucial health and environmental concerns [14]. Poor bioavailability of Zn from inorganic source [72] has encouraged nutritionist to search for mineral sources of higher bioavailability. Use of nanoparticles in nutrition is a thing of interest because of its ability to break cellular membrane barrier that might inhibit other forms because of sizes. Nanoparticles have shown novel properties such as higher bioavailability [10,60], higher surface activity, catalytic efficiency, and strong adsorption which is not peculiar to normal-sized particles and has higher uptake and absorption and capable of reaching body intricacies such as the tissue, lymph systems, liver, and spleen [73].

The study of Uniyal et al. [71] on zinc nanoparticles showed a 19% increase in serum Zn and 57.84% increase in erythrocytic SOD activity. Serum increase may be attributed to higher absorption of Zn, which would have come from bioavailable Zn while SOD activity showed increased ability of Zn nanoparticles to enhance cytointegrity. Nanoselenium supplementation (0.075, 0.15, 0.30, and 0.60 mg/kg diet) had higher erythrocyte SOD with 2.23-, 5.79-, 3.54-, and 4.89-fold increase than 0.3 mg/kg sodium selenite supplementation in laying birds [74].

Villus length, crypt depth, villi/crypt ratios, villus width, and villus surface area are important indicators of intestinal morphology, which play critical roles in nutrient absorption. A 30 nm–sized 1,200 mg/kg diet ZnO nanoparticles and 20 mg/kg colistin sulfate and 20 mg/kg colistin sulfate + 3,000 mg/kg ZnO showed that ZnO nanoparticles are capable of increasing gut structure, absorption, and regeneration by improving the villus length, crypt depth, and villus surface area of duodenum and ileum [75]. Surface area improved by 20% and 52% in duodenum and jejunum, respectively. The use of inorganic sources of minerals for farm animals is a challenge because it causes environmental pollution due to the low bioavailability of minerals, which results in 20–30 times the nutritional requirement of animals [71], and unabsorbed ones are excreted in feces and urine, which contributes to water or soil pollution. Nanoparticle use as mineral source may be a good option to reduce this contamination. Fecal output of ZnO nanoparticles was 37% lower than 20 mg/kg colistin sulfate plus 3,000 mg/kg ZnO [71]. This improvement in the gut structure with the use of nanoparticles as feed additives may be useful for nutrient absorption in equines.

4.4. Nanoparticle and Methane Emission

Nanomaterials are eco-environmentally sustainable and significant advances have been made in the field of green nanotechnology [76]. Nanoparticles have been reported to be toxic to certain microorganisms and inhibit the methane gas generation in animals with anaerobic digestion [15].

About 100 mg/L and 1,000 mg/L of cerium oxide and zinc oxide reduced methane output during anaerobic digestion by 27.8% and 79.1%, respectively, compared with control [77]. This is due to strong inhibition for hydrolysis and methanogenesis steps [36]. At concentration of 630 mg/L of cerium oxide, biogas production was 100% inhibited in anaerobic digestion [78]. This may be useful for equine manure management, which generates methane when managed inappropriately.

Short-term (8 days) and long-term (105 days) exposure to 30 and 150 mg/g total suspended solid ZnO nanoparticles reduced methane production by a 21.5% and 76.4% for 30 and 150 mg/g respectively, in short term, while 30 and 150 mg/g reduced methane by 19.8% and 73.9% respectively, in long term, which reduced the chance of adaptation [79]. This may be useful for in vivo reduction of methane emission and anaerobic production of methane through poor management of feces. The reduction may be attributed to lower population of archaea and the suppression of acetate kinase and coenzyme F420 [36].

The use of Cu nanoparticles showed a reduction in Cu excretion through the droppings of the animal [10,80]. There was 12.35%

Table 1
Summary of potential benefits of nanoparticles in equine.

Nanoparticles	Dose	Application	Effects	Reference
Citrate-reduced gold and biosynthesized silver	Not specified	Starch digestibility	Improvement of α-amylase activity to enhance starch digestibility	[39]
Citrate-stabilized gold nanoparticles	Not specified	Starch digestibility	Improvement of α-amylase activity to enhance starch digestibility	[42]
Zinc	60 ppm	Feed digestibility	improved activities of digestive enzymes (protease, amylase, and lipase)	[43]
Zinc oxide	30 ppm	Feed digestibility for energy generation	Improved gas production and short chain fatty acid from plant protein	[44]
Selenium nanoparticle	4 g/kg dry matter	Feed digestibility	Increased propionate acid, total volatile fatty acids, reduced ammonia nitrogen, improved dry matter and neutral detergent fiber digestibility	[46]
Magnesium oxide	0.7,1.0 and 1.4 mg/mL	Antimicrobial therapy	Antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa*	[58]
Copper nanoparticles	0, 25, 50, 75 mg/kg	Growth promoter, alternative to antibiotics	Improved growth rate, lower ammonia nitrogen, increased total bacteria, reduced population of *E. coli* and *Clostridium* spp.	[63]
Calcium, zinc, and silver nanoparticles	40 ppm	Antiperiodontitis	Reduced population of *Veillonella parvula*, *Porphyromonas gingivalis*, and *Streptococcus oralis* and weakening of biofilm formation	[53]
Silver nanoparticle	50 μg/mL	Antifascioliasis	Inhibition of egg hatching	[70]
Zinc oxide	30 and 60 mg/kg diet	Antioxidant, preventing inflammations, treating sport injuries	Increased superoxide dismutase	[4]
Zinc nanoparticle	20 ppm	Antioxidant	Enhanced erythrocyte superoxide dismutase	[71]
Nanoselenium	0.075, 0.15, 0.30, 0.60 mg/kg	Antioxidant	Enhanced erythrocyte superoxide dismutase	[74]
Zinc oxide	1,200 mg/kg diet	Gut health, mineral source	Improved villus length, crypt depth and improved villous surface area of duodenum and ileum, reduced fecal mineral output	[75]
Cerium dioxide and zinc oxide	100 mg/L of cerium and 1,000 mg/L of zinc oxide	Greenhouse gas mitigation	27.77% and 79.11% reduction in methane output	[77]
Zinc oxide	30 and 150 mg/g total solid	Greenhouse gas mitigation	21.5 and 76.5% reduction in methane output	[79]
Copper nanoparticles	25, 50, 75, and 100% replacement of standard copper recommendation, that is, 7.5 mg/kg	Mineral supplementation as replacement for inorganic source	Reduced fecal mineral output	[80]
Silver and magnesium oxide, and ferrous oxide Nanoparticle	500 mg/g total suspended solid, 100 mg/g Fe_2O_3	Greenhouse gas mitigation and probiotic	Reduced methane output and ferrous oxide improved bacteria growth	[81]
Cobalt nanoparticle	2 mg/L	Greenhouse gas mitigation	63.6% and 98.21% reduction in methane output than control and $CoCl_2$ salt	[82]
Fullerol $C_{60}(OH)_{24}$	10, 100, 1,000 ng/mL	Feed additive against toxins	Prevent synthesis of aflatoxins precursor	[90]
Honey-derived silver nanoparticle	10, 20, 30 and 40 μg	Feed additive against mycotoxins	Reduces aflatoxin production	[12]
Zinc oxide nanoparticle	2, 4, 6, 8, 10 μg/mL	Feed additive against mycotoxins	Reduced mycotoxin production and complete inhibition at 10 μg/mL	[93]
Silver nanoparticle	90 μg/mL	Feed additive against aflatoxin	Inhibition of aflatoxin B1	[94]

reduction in excretion compared with the standard inorganic Cu supplementation even when Cu nanoparticle was included at the recommended level of 7.5 mg/kg feed in poultry. This is important for environmental considerations. Hence, Cu nanoparticle may be included in the equine diet or used in premixes used in formulating equine diets.

Anaerobic digestion of sludge showed that 500 mg/g total suspended solid of Ag nanoparticles and 500 mg/g total suspended solid of MgO nanoparticles generated lower levels of methane production (73.52% and 1.08% than control, respectively) [81]. In addition, bacteria growth increased by 119% compared with control after exposure to 100 mg/g total suspended solid of Fe_2O_3 nanoparticles.

Abdelsalam et al [82] studied in vitro anaerobic digestion, with a similar occurrence of microorganisms compared with that of hindgut fermenter, to generate bioenergy through microbial fermentation of feed consumed. Results showed that inclusion of cobalt nanoparticles reduced methane and associated greenhouse gasses generated in biogas. Two milligrams per liter Co nanoparticles included in anaerobic digestion of slurry reduced biogas production by 38.56% and 76.56% compared with control and 1 mg/L $CoCl_2$ salt, respectively. Furthermore, 2 mg/L Co nanoparticles reduced methane output by 63.6% and 98.21% compared with control and 1 mg/L $CoCl_2$ salt, respectively. This was due to alteration in the atoms and development of a magnetic force. It can be projected that the smaller size of nanomaterial possesses a larger surface area and exhibits more activity [76]. Hence, cobalt nanoparticle has the potential to reduce methane if included in equine diets. This calls for further in vitro fermentation studies using equine feces output or rumen fluid.

4.5. Mycotoxins Removal

Mycotoxins are a byproduct of various fungi, especially *Aspergillus flavus* and *Aspergillus parasiticus* [12], which grow on grains either on the fields or during storage. These contaminated grains are eventually fed to livestock, which affects animal performance. Cereals grains are added to the equine diet to complement grass, hay, or silage. These mycotoxins can cause intoxications [83], can affect the athletic performance of horses, influence the reproductive system, and decrease the economic value of a horse [84].

Mycotoxins such as aflatoxins, zearalenone, deoxynivalenol, T-2 toxin, fumonisins, ochratoxin A, N-acetyl norloline, and peramine are prevalent in maize, silage, and finisher diets. They are capable of disrupting animal performance. Other emerging mycotoxins are fusaproliferin, moniliformin, beauvericin, and enniatins, claviceps (ergot alkaloids), and alternaria (altenuene, alternariol, alternariol methyl ether, altertoxin, and tenuazonic acid) [85]. These mycotoxins are among the most dangerous for animals. They may alter immune system such as immune suppression of inflammation [84] loss of feed intake, weight loss, reduced productivity, increased liver enzymes, cardiotoxicity, central nervous system disorders, gastrointestinal tract damage, nephrotoxicity, and hepatotoxicity [86], and its effect is more devastating in nonruminants than

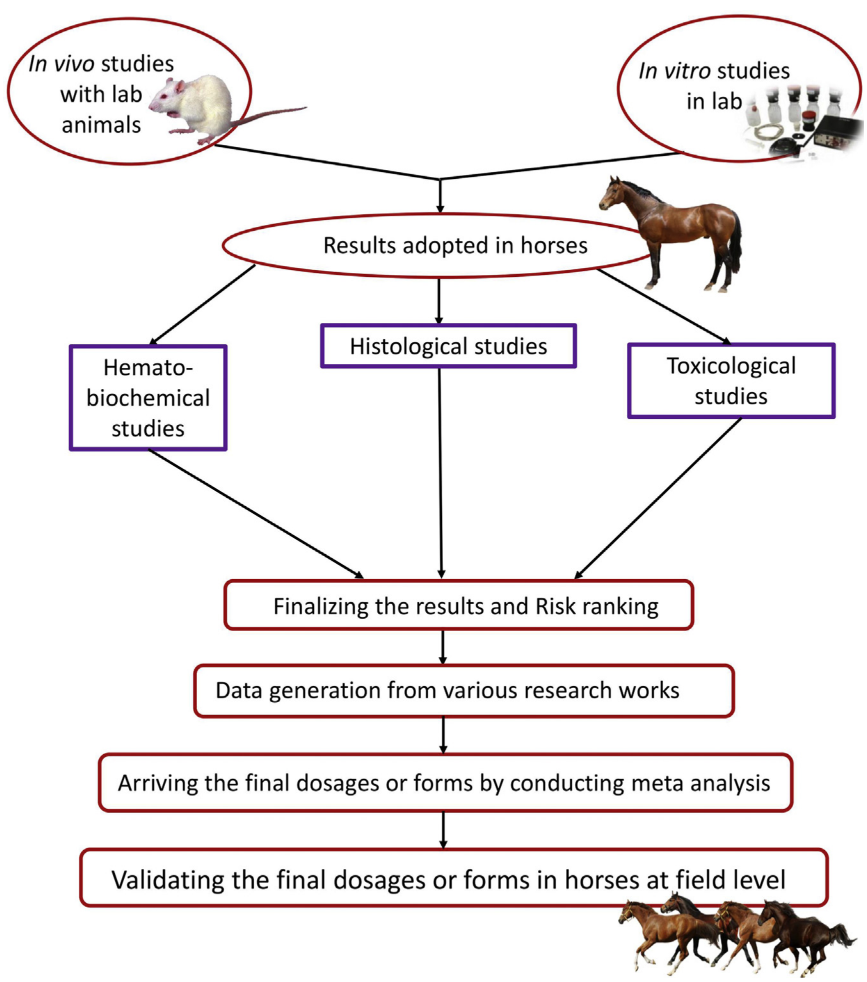

Fig. 2. Flowchart of the investigation methodology for validating nano-based feed additives in equines.

ruminants [87]. Various adsorbents such as yeast cell walls, clay binders, antioxidant additives [86] nanoparticles, and products from Biomin and Alltech are currently used as nutritional additives to bind or reduce the negative effect of mycotoxins in livestock. Nanotechnology approaches seem to be a promising, effective, and low-cost way to minimize the health effects of mycotoxins [86] including nanominerals and nanocomponents. The advantage of nanoparticles is due to their high surface area–to–volume ratio, which enables them to bind to mycotoxins [88].

It is well documented that there is a relation between oxidative and/or drought stress, conidia production, and aflatoxin biosynthesis [89]. Kovač et al [90] studied the influence of fullerol nanoparticles at 8 nm on mycelial growth of *A. flavus* NRRL 3251 in vitro; 10 g/mL resulted in mycelia growth suspension between 72 and 144 hours of the growth phase. Furthermore, all tested level of fullerol $C_{60}[OH]_{24}$ nanoparticles (10, 100, and 1,000 ng/mL) reduced the synthesis of norsorolinic acid, an aflatoxin precursor, by about 67% in 72 hours. The mechanism of biological activity of fullerol nanoparticles is related to their antioxidative properties, while *A. flavus* is sensitive to oxidative status perturbations [90]. The inhibition may be due to the interactions with and/or adsorption onto the fungal cell wall [91], where fullerol nanoparticles alter the fungal cell signal input by interfering with gene expression regulatory networks upstream of aflatoxin B1 biosynthesis or block a biosynthetic enzyme activity [92]. This implies that fullerol nanoparticles could be added to equine diets. However, confirmatory tests should be carried out in vitro.

A study showed that 1, 2, and 3 mg/100 mL media of honey-derived Ag nanoparticles of 9 nm reduced the aflatoxin produced by *A. parasiticus* by 21.79, 46.81, and 61.13%, respectively [13]. Similarly, 1–3 mg of 100 mL media of honey-derived Ag nanoparticle ochratoxin A by *Aspergillus ochraceus* was reduced by 45.83, 58.20, and 79.9% for 1, 2, 3 mg, respectively, and significantly inhibited the mycelium growth of both fungi in a dose-dependent manner when treated with Egyptian honey-Ag nanoparticle colloids at 10, 20, 30, and 40 μg. The inhibitory effect of Ag nanoparticles on fungal growth may be due to alteration of cell membrane permeability, the release of lipopolysaccharides and membrane proteins, generation of free radicals responsible for the damage of membrane, and dissipation of the proton motive force, which results in the collapse of the membrane potential [24]. Pretreatment of aflatoxigenic, ochratoxigenic, and FB1 fungi with ZnO nanoparticles at 2, 4, 6, 8, and 10 μg/mL reduced mycotoxins production, while 10 μg/mL inhibited it completely [93]. Furthermore, incubation of *A. parasiticus* with silver nanoparticles for 7 days at concentration of 90 μg/mL to determine aflatoxin production inhibited AFB1 production. Hence, the benefit of silver nanoparticles as a potential antifungal option is evident [94]—Table 1. The beneficial effects of feed additives observed in various livestock species could not be assured and effective in equines because of the dissimilarities in dosage requirements [95]. To arrive a safe dosage of nano-feed additives in equines, especially at the field level, requires an extensive investigation as shown in Fig. 2.

5. Conclusion

In the exploration of feed additives to improve equine production, nanoparticles offer a promising alternative because of its potential as probiotic, enhancer of digestibility, capacity to reduce greenhouse gas production, antimicrobial properties, enhancer of gut structure development, capacity to improve animal health, boosting of mineral absorption and capacity to reduce feed mycotoxins. Hence, nanoparticles application is a novel area for the equine industry and should be explored in greater depth.

6. Implication

The present review was conducted to provide information on the potential of nanotechnology in the equine research field. Hence, instead of antibiotics, metallic and synthesized nanoparticle components could be used as alternative antimicrobial agents against pathogenic microbes. Mycotoxin contamination of agricultural products used in equine nutrition could also be avoided by nanoparticles. Furthermore, poor starch digestibility in the stomach of horses could be enhanced by nanoparticles because of its ability to improve and prolong the activity of digestive enzymes, which could help to tackle digestive upsets relating to excessive grain feeding. Mitigation of greenhouse gasses in equine is an important area that nanoparticles could fix. Nanoparticles have the potential to resolve challenges in equines and could thus be included as feed additives in diets for horses.

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