Turkish Journal of Food and Agriculture Sciences

TURJFAS

6(1): 1-10 (2024)

Review

DOI: https://doi.org/10.53663/turjfas.1394943

A new approach to the horse nutrition: Nanoparticles

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ARTICLE INFO

HISTORY

Received: 23 November 2023
Revised: 6 January 2024
Accepted: 30 January 2024
Online Published: 30 June 2024

KEYWORDS

Alternative feed additives
Horse nutrition
Nanoparticles

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ABSTRACT

There has been a gradual increase in research on alternative feed materials and feed additives in animal nutrition. Since the purpose of animal nutrition is to ensure healthy and sustainable animal production, the primary objective is to ensure that the alternative substances are not only beneficial to disposal of waste, but also to the health and development of the animals. Particularly in horse farming, feeding is based on commercial diets supplemented with some vitamin additives. However, the specific digestive anatomy and physiology of horses create obstacles in the methods, which used to compensate for deficient feedstuffs and nutrients. Nanoparticles, which are widely used especially in human nutrition and discovered in search of alternative sources after various legal regulations in animal nutrition, have not yet opened a field for itself in equine nutrition. In this study, the aspects and possibilities of using nanoparticles, which are frequently used in ruminant and poultry nutrition, in equine nutrition were discussed and the pros and cons of nanoparticles were criticized.

Citation: Evci, Ş. (2024). A new approach to the horse nutrition: nanoparticles. *Turkish Journal of Food and Agriculture Sciences*, 6(1), 1-10.

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1. Introduction

Horses, which have been in people's lives since ancient times, started to be raised for sportive purposes in developed countries following the Industrial Revolution. Feeding in horses raised for sportive and hobby purposes has been shaped depending on parameters such as the physiological state, age, gender and performance level of the horse and has become almost traditional. Misformulated horse diets lead to serious issues by causing some metabolic problems and this situation is negative for both horse health and the benefits of the producer.

Nanoparticles are the substances that contain at least 50 % of natural substances and vary in size between 1 to 100 nanometers (Garcia-Barrasa et al., 2011). The gradual development of nanoparticles has led to the expansion of their use in animal nutrition. Nanoparticles can be used as feed additives instead of antibiotics which are banned due to residue risk and microbial resistance (Reddy et al., 2020). Some nanoparticles (e.g. nanozinc and nanosilver) are being considered potential alternatives to antibiotic feed additives because of their bactericidal capabilities. Moreover, unlike their macro counterparts, the fact that nanoparticles reduce mineral excretion and environmental pollution has played a role in the evaluation of their usability as feed additives (Gopi et al., 2017). The total dose required to achieve competent serum concentrations is lower for nanoparticles than for non-nanoparticles. This allows the desired level of nutrition to be achieved by using fewer additives (Wang et al., 2018). The application of nanotechnology in animal nutrition, specifically through the use of nanoencapsulation and nanoparticles, allows for precise and minimal addition of materials while still achieving the same function as their bulk-sized counterparts. This is commonly referred to as 'nano nutrition' or 'nano additives'. Research in animal nutrition has utilized metal and phyto-based synthesized nanoparticles, including silver, gold, calcium, iron, selenium, silicon, titanium, and zinc, in various fields of nanotechnology (Adegbeye et al., 2019).

Although there are various studies on the use of nanoparticles in livestock species, they are generally limited to ruminants and laboratory animals. The use of nanomaterials in equine nutrition has been mostly related to preventive or therapeutic medicine (Gopi et al., 2017; Elghandour et al., 2018; Xie et al., 2018; Adegbeye et al., 2019). The use of nanoparticles as feed additives, particularly in immunotherapy, is supported by their easy digestibility through the intestinal lumen (Hameed, 2021; Hill and Li, 2021). Polymeric, liposome, dendrimer, micellar, and ceramic nanoparticles are commonly used in animal production and feed additives. Carbon-based nanoparticles, such as fullerene and carbon nanoparticles, as well as metallic inorganic nanoparticles, have also been used. Among the metallic nanoparticles, gold, silver, cobalt, copper, chromium, and magnesium are frequently employed in livestock production. Metallic nanoparticles, including gold, silver, cobalt, copper, chromium, magnesium oxide, ferrous oxide, zinc oxide, titanium oxide, and selenium, are frequently utilized in livestock production. It is important to note that this statement is objective and does not contain any subjective evaluations (Adegbaye et al., 2019;

Nanoparticles commonly induce oxidative damage by generating reactive oxygen species, disrupting cell membranes, and inhibiting cell division and death (Zhu et al., 2007; Rudramurthy et al., 2016). The formation of excessive reactive oxygen species, including hydrogen peroxide, leads to oxidative stress and subsequent cell damage (Allen et al., 2009). Other effects include depletion of intracellular ATP production and disruption of DNA replication (Donato et al., 2010).

Nanomolecular additives such as gold nanoparticles, iron-oxide nanoparticles, gelatin nanoparticles are frequently used to improve performance in horses (Reddy et al., 2020). This group includes gut-floral stabilizers, digestibility enhancers and environmental modifiers and other additives that improve the nutritional status of horses. The beneficial effects of antibiotics used as feed additives in various livestock species have not been achieved in horses due to differing dosing requirements (Elghandour et al., 2018). Because of the capability of metal nanoparticles to disrupt gram-positive and gram-negative bacterial cell walls, nanotechnology is considered as an alternative to several antibiotic feed additives (Xie et al., 2018). Bacterial (e.g. Clostridium perfringens, Clostridium difficile, Escherichia coli, Bacteroides fragilis, Enterococcus, and Aeromonas spp.) foal diarrheas are highly contagious infections and negatively affect growth performance (Mallicote et al., 2012).

Mallicote et al. (2012) also reported that 800 mg kg^{-1} nano-ZnO supplementation to the diet decreased diarrhea rate and positively affected daily body weight gain in foal diarrhea. Spherical silver nanoparticles are also known to decrease diarrhea incidence through bacteriostatic and bactericidal effects on Salmonella and Shigellas (Tiwari et al., 2018). Although, according to the literature review, there is no similar study conducted in adult horses, the reported results for foals suggest that similar effects can be achieved in adults by adapting these substances. In this study, various empirical researches and comprehensive literature reviews on the use of nanoparticles in nutrition of horses were reviewed.

2. Production of nanoparticules for animal feed additivities

Nanoparticles can be synthesized in the laboratory or purchased commercially. Nanoparticles can be produced using traditional chemical and physical methods or using green and sustainable biosynthetic methods that use plant extracts or microorganisms (Gopi et al., 2017; Yusof et al., 2019; Abdelnour et al., 2021). The methods used to obtain nanoparticles and their classification are given in Table 1.

Table 1. Production methods of nanoparticles (Michalak et al., 2022)

Physicial Methods	Evaporation and condensation, Physical Evaporation, Chemical Deposition, Electric Arc Discharge (EAD), Ballmilling-Annealing, Gas Phase SynthesizingLaser ablation
Chemical Methods	Reduction methods Chemical Indirect
	Sedimentation methods Sol gel, alkaline, and co-precipitation hydrothermal
	Inert gas condensation
Biological Methods	Using plants, bacteria, virus, fung, etc.

Microencapsulation techniques involve physically entrapping sensitive bioactive compounds in a matrix of macromolecules, allowing for safe delivery through deleterious environments until assimilated in the proper organs (Galland, 2013). However, this method's large particle size does not solve the problem of low bio-accessibility of the material after digestion and release processes. In this context, nano-encapsulation could be an effective approach to increase the bioactivity and bio-accessibility of the compounds, as well as to enhance their stability under digestive conditions and protect them from interacting with other components of digestion and premature degradation before reaching the target site (Neilson et al., 2007). Nano-encapsulation enhances cell permeability and solubility during the digestion process, resulting in proper absorption and bioavailability of nano-encapsulated compounds (Hu et al., 2002). Polysaccharides, including starch and its derivatives, pectin, glucans, cellulose, and protein-based materials such as polypeptone, soy protein, milk proteins, and gelatin derivatives, are the most widely used materials for encapsulation in food or pharmaceutical applications (Ahmad et al., 2017; Ahmad et al., 2018).

3. Using nanoparticles as feed additives

Feed additives can be defined as the substances that improve the digestibility, utilization or storage of basic diet components (Elghandour et al., 2018). Primary components of the diets (i.e. amino acids, vitamins, minerals, and poly-unsatureted fatty acids (PUFA)) are used for direct consumption in small-scale quantities. Nanoparticles are also under investigation in the food industry as a delivery system for nutraceuticals. Because of the different gastrointestinal tract of horses compared to other livestock species and the different pH levels between parts of the digestive tract, feed additive or nutraceutical evaluation is mandatory. Nanocarriers have shown good results in delivering nutrients for efficient absorption at the small intestine (Singh et al., 2017). Cause of their ability to emulsify and their well gelling properties, proteins are considered to be safe nanocarriers. An in vitro release study simulating the gastrointestinal tract displayed that the cruciferin (i.e. canola protein) is a prospering carrier for beta-carotene. It is released at intestinal pH (Akbari and Wu, 2016).

It is known that processed cereals used in horse feeding are subjected to processing-related loss of many vitamins and minerals besides causing some metabolic disorders (Reddy et al., 2020). Bioavailability is directly related to bioaccessibility, absorption and molecular transformation (Salvia-Trujillo et al., 2016). Therefore, delivery systems on the basis of nanomaterials increase nutrient bioavailability. Draught and racehorses have a higher physiological need to nutritional supplements. However, because of the other elements that interfere with absorption, inorganic mineral salts have poor bioavailability. The addition of these inorganic minerals with low utilization levels to horse feeds leads to increased excretion rates and environmental pollution. The use of nano-sized minerals can increase bioavailability by reducing the amount of these waste materials.

The expectation of improved performance as a result of increased absorption rates of nutrients with the use of mineral nanoparticles is related to this physiological transformation. The increased surface area and the reduction in size of the mineral nanoparticles lead to improvements in certain physico-chemical characteristics (Boyles et al., 2016). The nanoencapsulation technique used in the delivery of vitamins A, C and E to body tissues has been developed by utilizing this capability. In addition to the increase in absorption and diffusion efficiency, the nano-encapsulation technique also contributes to the reduction of inflammation and acceleration of the recovery process (Bunglavan et al., 2014). Although all these physiological and biochemical processes have been tested in vivo and in vitro in different livestock species, the literature on their use in horses is quite limited.

Although there are very few nano-elements that have ever been studied in equine nutrition, the most widely studied is the nanoform of vitamin E (Ezhilarasi et al., 2013; Bunglavan et al., 2014; Sinatra et al., 2014). To support optimal neurological and muscular activity in performance horses, an adequate intake of vitamin E is necessary. Readily available commercial forms of vitamin E are commonly recommended for expectant or nursing mares, stallions, racehorses and convalescent grown horses. Coenzyme Q10 (CoQ10), being an indispensable nutrient for mitochondrial energy generation, is also a nanosuspension besides vitamin E. Nonetheless, CoQ10 is barely bioavailable because of its low water solubility. The tenside nanosuspensions cause CoQ10 enzyme constituents to be more soluble and bioavailable (Sinatra et al., 2014). Therefore, excepting in case of ubiquinone deficiency, CoQ10 suspensions, which can collaboratively function with vitamin C and vitamin E, are often used to combat oxidative stress in racehorses. Casein micelles are widely regarded to be natural nanoparticles. They bind to proteins, calcium and other nutrients to enable them to be transferred from expectant mother to her foal. Casein nanoparticles are described to be effectual in delivering oleophilic nutrients and they can increase growth rates by facilitating nutrient delivery in weaned foals (Hill et al., 2017).

Nanostructured MgO is a low-cost and easily manipulable nanoparticle that exhibits intrinsic biocompatibility (Auger et al., 2018). It has been shown to possess antimicrobial activity against Escherichia coli and Pseudomonas aeruginosa at concentrations of 0.7, 1.0, and 1.4 mg/mL by damaging the cell wall, cell membrane, and destroying formed biofilms (Nyuguen et al., 2018). Additionally, these nanoparticles may be used as prebiotics in animal feeding (Fondevila et al., 2009). Similarly, the use of copper-loaded chitosan nanoparticles results in an increase in the population of Lactobacillus and Bifidobacterium in cecal digesta, as well as a decrease in coliforms population (Wang et al., 2011). Additionally, supplementation with graded levels of 0, 25, 50, and 75 mg/kg copper nanoparticles leads to an increase in the growth of total bacteria, ranging from 45.97% to 105% compared to the control (Refaiye et al., 2015). These findings suggest the potential probiotic benefits of nanoparticles. Furthermore, the levels of pathogenic *E. coli* and *Clostridium* spp. were reduced by approximately 1.6- to 2.7-fold and 1.37- to 2.86-fold, respectively, compared to the control group when supplemented with nanoparticles (Adegebeye et al., 2019).

Ruminant feeds containing urea may be tolerated to a limited extent. The NPN compounds can be supplemented into horse diets at specific amounts, as urease activity in the equine caecum is approximately 25% of that in the rumen for ruminants (Martin et al., 1996). Nevertheless, horses are known to have a narrow range of tolerance to urea, around 15% of plasma urea levels, so excessive use may lead to undesirable results. Although urea feeding to mature horses with marginal protein intake is not widely recommended because it may have beneficial effects in terms of feed cost, it is often used in practice (Martin-Rosset and Tisserand, 2004).

However, excretion of unused nitrogen results in a significant environmental nitrogen load (Reddy et al., 2019a; Reddy et al., 2019b). Kottegoda et al. (2017) proved that urea release was reduced by including it in hydroxyapatite nanoparticles. It can be concluded that by delivering urea with the mentioned nanoparticles, the protein deficit in the basal diet can be completed and a safer and healthier feeding can be achieved in horses.

Equine stomach cannot fully digest starch. Therefore, when the incompletely digested starch enters the caecum-colon chamber (wherein anaerobic fermentation region), as a result of hindgut environmental pH depression, usual microbial balance and/or microbial activity is disrupted. Consequently, digestive disorders in horses are associated with diets containing high starch grains (Cipriano-Salazar et al., 2019). However, increased stomachal carbohydrate digestion results in a reduced starch flow into the hindgut. In this case, yeasts are often used to help, but nanoparticles are known to function in a similar way (Adegbeye et al., 2019). Nanoparticles can also be safely added into equine diets for a better starch digestibility. The activities of enzymes involved in digestion (e.g. amylases, lipase, protease) can be regarded as hallmarks of nutrient utilization capability, and to some degree, digestibility based on the diet provided (Gomez-Requeni et al., 2013).

It was revealed that alpha-amylase activity increased in coexistence with citrate-reduced gold and biosynthesized silver nanoparticles (Saware et al., 2015). While the coexistence of gold and silver nanoparticles resulted in a 1.5-fold increase in α-amylase activity, gold nanoparticles alone brought about higher activity. Subsequent researches indicated that a higher starch solution resulted in a higher enzymatic activity. In the presence of nanoparticles, there appears to be an increased breakdown of starch into sugars, resulting in a catalyzing effect (i.e. nanocatalyzing effect) on starch uptake, more likely. This nanocatalytic activity may reduce starch granules flowing into the hindgut of horses, and therefore microbial dysbiosis. The immobilized enzyme overrides the common collision frequency between free enzymes and substrate molecules, and then the enzyme binds to the nanoparticles instead of the reducing sugars, albeit the enzymatic activity still sustains (Jiang et al., 2005). This may also be administered to fibrolytic enzymes in equine feeds to increase fiber digestibility. On the other hand, depending on the enzyme activity, it may be necessary to use lower levels of fibrolytic enzyme in the horse feeds. Likewise, it was observed that averagely 11 nm-sized gold nanoparticles (stabilized with citrate) resulted in higher alpha-amylase activity with incrementing starch concentration (Deka et al., 2012). In the presence of gold nanoparticles in the medium and when probed with starch, the concentration of α-amylase is found to be 5.5 to 9.6 times higher, approximately. The unexpected lower enzymatic activity accompanied with the higher alpha-amylase is a consequence of binding of the enzyme to the nanoparticles and following agglomeration (Gosh et al., 2013).

One of the feed additive groups is sensory additives. Sensory additives enhance animal palatability and appetite by providing sensory attitude of aroma, palate and odor. Food flavorings are often used in commercial horse diets to combat with hesitancies against any nutritional novelty (neophobia) and encourage the consumption of tasteless additives, water and anthelmintics.

It was shown that, tasteless pellets, when they were aromatized with fenugreek or banana, the feed intake is promoted (Goodwin et al., 2005). Although never tested previously in horses, it is well documented that, some common nanoencapsulation methods result in increased aroma release in foods. The capability of silicon dioxide nanomaterials to carry odors has been demonstrated empirically for food applications (Malheiros et al., 2010; Bokkers et al., 2011). Malheiros et al. (2010) revealed that nano-based liposome entrapment method provides more effective delivery of flavorings rather than other encapsulation methods. This is a method that contributes to the provision of basic flavors in horse feed.

It is well documented that feed additives often play a role in the improvement of animal health. Therefore, nanoparticles are also used as feed additives in the prevention of protozoal diseases observed in horses (Raguvaran et al., 2015; Dubey and Bauer, 2018).

An in vitro study showed that silver nanoparticles (4.6 nm in size) produced by *T. harzianum* reduced Fasciola hatchability by 28.71% compared to conventional triclabendazole use (Gherbawy et al., 2013; Wu et al., 2016).

4. Using nanoparticles in feed hygiene

Technological methods are constantly used to ensure the hygiene of feeds or feed additives. Substances used for feed hygiene may include antioxidants, preserving agents, emulsifying agents, stabilizing agents, silage additives and acidity regulator. Developments in the food science and technology have paved the way for the study of nanoparticles being biocides to preserve feeds (Hill and Li, 2017). Contaminations that may occur in feeds and especially mycotoxins can have serious negative impacts on animal health (Kottegoda et al., 2017). The use of plastic containers implanted with silver nanoparticles can minimize the risk of contamination with their antimicrobial properties (Bunglavan et al., 2014). Although it has been suggested that metal-based nanoparticles are transferred from feed containers, on the other hand recent studies have shown that silver nanoparticles in metal-impregnated feed boxes are untraceable. Therefore, the use of multiple silver-based zeolite products for disinfection purposes has been certified by the US Food and Drug Administration bureau (Reddy et al., 2020). Another study revealed that mycotoxin contamination is very common in equine feed mixtures sold in the market. Although determined mycotoxin concentrations were below the toxicity threshold level, it was found that they caused skin allergies and various inflammations; as the accumulation level increased, they caused serious health problems (Horky et al., 2018). The use of nanoparticles that can bind to toxins in reducing or completely preventing toxicity can be considered as a critical progress in terms of animal health (Ajdary et al., 2018).

Propionic acid is a common supplement to reduce dust and mold, and to extend expiry date in equine feeds. The conservatory nanotechnology products (e.g. chitosan films containing silicon dioxide nanocrystals, alginate nanolaminate coatings, gelatin-based cellulose nanocrystals, and nano-silica coatings) seem to be more hopeful method rather than the rest of the conventional methods to sustain feed quality even along an extended shelf-life (He and Hwang, 2016). Aluminum is preferred to prevent caking of horse feeds during storage, but it is also known to have a negative effect on phosphorus absorption. Although there is no alternative product or substance yet, the European Union has registered nano-sized silicon dioxide being an anti-caking agent; on the other hand, it has not yet been validated in horse feeds (Ezhilarasi et al., 2013). It is well documented that the vast majority of the nanometallic particles generate oxidative stress through the release of ROS (reactive oxygen species), and this is one of the negative characteristics of nanoparticles. However, a small number of reactive nanomaterials (e.g. polymer coatings and silicon dioxide gallic acid) are used to deliver antioxidants in the food industry (Horky et al., 2018). Lipid-based nanoencapsulation systems, which contribute to increased particle solubility and bioavailability, may result in higher antioxidant performance (Wolny-Koładka and Malina, 2017).

5. Pros and cons of nanoparticles

The narrow safe and toxic dose range of nanoparticles may cause some concerns in the use of nanoparticles. There is a need for safe studies on the use of nanoparticles in equine nutrition, as this is an area that has not yet been adequately studied. When used as feed additives, the interaction between nanoparticles and diverse biological compounds should be well understood. Within this framework, the evaluation of the haemolytic capacity of zinc oxide nanoparticles showed that clustering depends on the concentration in horse erythrocytes (Raguvaran et al., 2015). Empirical studies are still a long way from determining exactly the appropriate dose. Since the digestive system in horses is very sensitive, and therefore reacts quickly to any change in the favorable flora (particularly *Lactobacillus* equi species flora). These bacteria additionally counteract specific enzymatic functions of the microbial flora in the equine large bowel and pro-carcinogenic bodies produced during digestive processes (Tiptiri-Kourpeti et al., 2016). It is not yet fully understood whether antimicrobial nanoparticles act on these favorable bacteria. Therefore, they need to be properly investigated before they can be used as a supplement in horse feeds. Discrepancies in disclosed results are another issue to be dissected. In an in vitro study, silver nanoparticles were found to be efficacious against coliforms without any adverse effect on Lactobacilli species (Fondevila et al., 2009). Contrarily, in another study it was revealed that the same compounds had a negative effect on Lactobacilli species but not on the pathogens (e.g. *E. coli*, and *S. aureus*) (Tian et al. 2018).

6. Conclusion

Nowadays, nanoparticles are increasingly used in ruminant and poultry nutrition, and ideas for their use in horses not only in health applications but also as feed additives are promising. Results supported by in vitro and in vivo applications show that the use of nanoparticles in horses can contribute positively to both digestive system health and physiological satiety. Considering the importance of waste management in sustainable livestock production, the fact that nanoparticles scarcely contribute to environmental pollution, and also costs and alternative protective aspects, the use of nanoparticles in horse nutrition can be considered as a new era. It is believed that with the studies on the use of nanoparticles in animal nutrition, the cost analyses will reach realistic dimensions. Although it is considered that these materials, which can be used in small quantities and can contribute positively to health, do not have an economic production considering the economic value of the horse today, the price analyses will be updated depending on the development of materials and methods to be used in the synthesis of nanoparticles with further studies. However, the physicochemical properties and biochemical effects of nanoparticles need to be further investigated, considering the scarce number of studies on this topic, and the inconsistency of the previous reports.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

Şevket EVCİ: Conceptualization, methodology, investigation, data curation, writing – review & editing.

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

We humbly give consent for this article to be published.

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