# Journal of Agricultural Sciences Research

### NANOTECHNOLOGY FOR MODERN SUSTAINABLE LIVESTOCK PRODUCTION

#### Bulmaro Méndez-Argüello

Facultad Maya de Estudios Agropecuarios. Universidad Autónoma de Chiapas. (FMEA-UNACH) Catazajá, Chiapas, Mexico

#### Ricardo Hugo Lira-Saldivar

Department of Biosciences and Agrotechnology, Research Center for Applied Chemistry (CIQA), Saltillo, Coahuila, México

#### Froylan Rosales-Martínez

Facultad Maya de Estudios Agropecuarios. Universidad Autónoma de Chiapas. (FMEA-UNACH) Catazajá, Chiapas, Mexico

#### Santa Dolores Carreño-Ruiz

Facultad Maya de Estudios Agropecuarios. Universidad Autónoma de Chiapas. (FMEA-UNACH) Catazajá, Chiapas, Mexico

#### Rubén Monroy-Hernández

Facultad Maya de Estudios Agropecuarios. Universidad Autónoma de Chiapas. (FMEA-UNACH) Catazajá, Chiapas, Mexico

#### Abisag Antonieta Avalos Lázaro

Facultad Maya de Estudios Agropecuarios. Universidad Autónoma de Chiapas. (FMEA-UNACH) Catazajá, Chiapas, Mexico



All content in this magazine is licensed under a Creative Commons Attribution License. Attribution-Non-Commercial-Non-Derivatives 4.0 International (CC BY-NC-ND 4.0). Abstract: At the beginning of the XXI century, nanotechnology (NT) is an emergent knowledge focused on characterizing, preparing and using nanoscale sized materials among 1 to 100 nanometers (nm). In the present review, emphasis will be directed towards NT applications in veterinary medicine and animal nutrition, especially on pork and poultry production. It is a well-known fact that trace minerals in their nanoparticle form are used to increase efficiency in animal nutrition, especially when used to modify ingredients to improve their flavor, palatability, color and texture; as well as to increase nutrients bioavailability. The use of nanoparticles (NPs) in veterinary medicine can eliminate biological barriers and promote the recovery of animal's health, due to the controlled release of drugs, which makes NPs an efficient alternative to antibiotics. In addition, NT has become a useful tool for noninvasive gamete bioimaging and nanopurification. It has also been used to develop protectors for cryopreservation, to optimize and eliminate reproductive obstacles.

**Keywords:** Nanoparticles, animal nutrition and production, veterinary medicine.

#### INTRODUCTION

In a relatively short period NT has gained significant impact in a broad range of sectors, including agroindustry, being focused on the characterization, preparation and application of nanoscale (1-100 nm) materials, thus located in the atomic and molecular range (Hameed, 2021). NT is used in many aspects of our daily life, such as in industrial products, medicine supply systems, disease diagnosis and treatment, and also in animal nutrition (Zhao et al., 2017).

The uses of NT are vast, including nutritious nanoadditives for animals, medicine for cattle, and antimicrobial nanoemulsions to treat and prevent animal diseases in farms,

as well as components for food packing (Elgendy and Zontahy, 2022). Modernization, industrialization and larger population has resulted in generation of huge amount of waste, with rich in organic and inorganic content. Waste biomass (agriculture, algae, sludge, livestock manure) are available in large quantity worldwide (Arun et al., 2022).

Cattle farming is an important part of the economy of most countries worldwide. Animal diseases significantly reduce animal growth, yield and quality of meat, milk and eggs. Therefore, significant scientific efforts have been made to create new diagnostic techniques that help identify and treat animal diseases. NT can improve disease diagnosis and treatment, as well as increase production of animal protein for the entire world population (Prasad et al., 2022).

Nanomaterials (NMs) in animal nutrition and veterinary medicine have been barely investigated; however, their use is increasing, and it is predicted to rise even more in the near future. NPs are now being used to efficiently supply nutrients, and as new generation antimicrobials. Minerals like selenium, copper, iron and zinc in nanometer size, when added to animal diets, can increase their absorption and bio utilization in such a way that they promote animal growth and improve meat quality (Hill and Li, 2017). Considering all the previously described facts, the objective of this review is to provide updated information on the different uses of NT in livestock production.

#### NANONUTRIENTS ON ANIMAL PRODUCTION

Minerals play an important role in animal growth and productivity. Adding minute amounts of mineral NPs to the diet improves nutritional quality. NT can help obtain detailed information about the location of certain nutrients in the tissues, cells or cell compartments. These technologies can improve animal nutrition and increase the nutrients bioavailability in the tissues (Thulasi et al., 2013). NT in animal nutrition includes modifying ingredients to improve their flavor, palatability, color and texture; as well as to enhance bioavailability of essential elements (Figure 1).

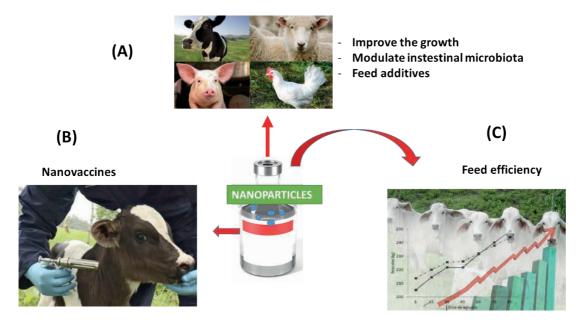


Figure 1. Some nanotechnology applications in domestic animal nutrition. (A) Some nanoparticles have very important applications in animals; (B) Nano vaccines are used to control certain diseases of animals; (C) Nanoparticles are used as nanonutrients to increase animal production.

Lately many strategies have been tried to reduce mineral supplementation of farm animals, especially of broiler chickens. NT is one of these strategies, because it significantly increases the bioavailability of minerals; for example, calcium. Minerals as NPs have an increased surface and contact area, which explains their improved absorption and utilization (Ganjigohari et al., 2017). NPs have high temperature and pressure stability and are easily absorbed in the animal's digestive tract. Therefore, NMs are more efficient at lower levels than their conventional forms, which allows to use only the necessary amount and reducing losses. Due to their small size and unique physical properties such as greater biological reactivity and greater contact area, NPs have an improved bioavailability and interaction with other nutritional components that significantly enhance animal growth and

production (Swain et al., 2015).

Zinc (Zn) is the second most abundant trace element in the body of broilers chickens. Zn cannot be stored in the body and thus must be supplied in the diet. On the other hand, current research show that NPsZnO not only promote growth, but can also act as antibacterial agents, modulate the immune system and have special functions in animal reproduction. Nano-Zn used at low doses has better results compared to conventional Zn sources. However, research on the optimum doses, bioavailability and period of supplementation is still needed, since nanominerals have the capacity to pass the small intestines easily and be distributed faster into the blood and organs, like brain, lungs, heart, kidneys, spleen, liver and stomach (Swain et al., 2016).

A study carried out with broilers, the effects

of different levels of silver NPs (AgNPs) in the diet on the intestinal microflora, carcass characteristics and blood parameters, were evaluated. Chickens were fed 2.5, 5, 10 and 20 mg of AgNPs per kilo of feed. The researchers found that 2.5 mg AgNPs improved growth and fattening of the animals. Although with high doses there was an accumulation in the tissues and organs (Al-Sultan et al., 2022).

Tilmicosin (TLM) is a semisynthetic antimicrobial agent used mainly in poultry and cattle, but it has a relatively poor oral bioavailability. Rassouli et al., 2016 compared bioavailability and the main pharmacokinetic parameters of TLM after oral administration of tilmicosin phosphate and three newly prepared lipid nanoparticles (LNPs) of TLM, including solid lipid NPs, nanostructured lipid carriers, and lipid-core nanocapsules. The authors concluded that TLM-LNPs improved the drug's bioavailability and its pharmacokinetic parameters, especially TLM lipid-core nanocapsules, which suggests an efficient delivery system for TLM.

NT has many applications in food processing and preservation. The basic food components like proteins, fats and carbohydrates are becoming more important in nano-engineering. NPs transport functional ingredients and provide additional value to food products; for example, formulated dairy products with nano-encapsulated nutrients influence texture, flavor and nutritional quality. This is important when feeding recently weaned animals (pigs and calves) that require a special diet to help them get used to a solid diet, in which sorghum, wheat and maize are incorporated (Chavada et al., 2016).

Gonzales-Eguia et al., (2009) observed that nano-copper could improve the digestion of energy and fat by increasing lipase and phospholipase A activity in the small intestine, compared to a diet supplemented with copper sulfate (CuSO<sub>4</sub>). Piglets showed an increase in their daily weight gain and a better immune capacity. The analysis of the influence of nano-copper in the immune system showed higher concentrations of total globulin and superoxide dismutase in blood serum. These results suggest that the nutritional value of foodstuff for domestic animals can be significantly improved using metal NPs.

The use of nano-capsules to cover and protect particular enzymes and proteins in animal diets can help to increase growth and yield (Gangadoo et al., 2016). Copper is regularly incorporated to animal fodder due to its capacity to promote animal growth and yield, and because of its antimicrobial properties. It was reported that copper NPs pass the intestinal mucosa easier than conventional copper, which leads to a better absorption (El Basuini et al., 2017).

Mohammadi et al. (2017) studied the effects of iron oxide NPs covered with L-cysteine for the reproductive yield in quails. The results indicate that the fertility of animals improved when NPs were added to their diet, finally leading to higher chicken weight. The results of this study show that NPs improved bioavailability and utilization of dietary iron, which increased the production and quality of eggs, as well as the feed conversion. Yang et al. (2014) added nano-vitamin  $D_3$ , to the diet of laying hens, and found higher egg production and quality with the addition of nano-vitamin.

Essa et al. (2017) studied a MgOSiO<sub>2</sub> nanocompound as an efficient absorbing agent to eliminate aflatoxins in wheat flour for animal diets to achieve the same goal, nanozeolite compounds were used to reduce aflatoxins in broiler diets (Shabani et al., 2016). Results reported by Khah et al. (2015) suggests that dietary no-NPs with 60 and/ or 90 mg/kg ZnONPs, improved quality of carcass broiler during the starter period.

#### NANOTECHNOLOGY AND VETERINARY MEDICINE

NPs inveterinary medicine help to eliminate biological barriers and to improve recovery efficiency. NPs promote drug effectiveness through controlled delivery and release. NPs are a viable alternative to antibiotics and can help to prevent lesions caused by pathogens, and reduced production of meat, milk and other by-products (Hill et al., 2015).

Positively charged metal NPs can damage the negatively charged bacterial membranes, leading to filtration and bacterial lysis (Gahlawat et al., 2016). The study by Mohamed et al (2017) found that NPsAg could inhibit the growth of Corynebacterium pseudotuberculosis. On the other hand, results by Farzinpour and Karashi (2013) point out that NPsAg caused significant decrease of yolk weight and hen-day egg production per week when compared to the control, whilst NPsAg had no significant effect on the weight, length and width of the egg and thickness of the eggshell. Silver is applied in medicine to prevent infections and to eliminate or inhibit the growth of certain pathogenic microorganisms (Elkloub et al., 2015).

Silver nanoparticles are known for their unique physical, chemical and biological properties. They are used as antimicrobial agents, although researchers have recently discovered other applications. For instance, adding 15  $\mu$ g/egg of NPsAg after 18 days of incubation will modulate the post-incubation immune response, without affecting the hatchability, growth and other yield parameters of broiler chickens (Goel et al., 2017).

In ruminants like sheep, functional peptides with certain inert NPs stimulate the immune response in cells (Greenwood et al., 2008). Carbon nanotubes (CNT), when introduced to the bloodstream, accumulate near the tumor or damaged region. When the animal is exposed to infrared light, CNTs slightly absorb the emitted energy, heating up to 55°C, and helping in this way to destroy the tumor without damaging other tissues (Coppo, 2009). The use of CNTs underneath the animal skin shows real time estrus and peak estrogen concentrations which allows to perform artificial insemination at the right moment and with a higher efficiency (Mousavi and Rezaei, 2011).

In veterinary medicine, vaccines are developed to achieve the overall well-being and health of animal species. If vaccines are delivered through the respiratory mucosa, this will reduce costs and diminish the dose, achieving a similar or even higher immune stimulation, with specific IgA antibodies, than the one obtained by conventional injection (Zinsstag et al., 2011). However, the specific mechanism between NPs and biological membranes is still being investigated, though it has been pointed out that the size and form of NPs, as well as their distribution, affect the release and protection of antigens at a specific site (Calderón-Nieva et al., 2017).

application The and efficiency of antibiotics can be improved by using NPs as carriers, which could reduce the dose used to treat certain infections. NPs targeting gram negative bacteria are the most important ones in animal production, since these bacteria are the ones that colonize in foodstuff and the digestive tract, and can cause multiple diseases (Smekalova et al., 2016). NPs functionalized with monoclonal antibodies can help to quickly and specifically detect certain kinds of viruses, compared to traditional detection techniques. In some studies, NPs have proven to efficiently manage the supply of antimicrobial agents, painkillers and antiinflammatory agents (Xing et al., 2016).

Nanostructured material, including NPs and nano-emulsions, are being used to develop new immunomodulatory agents, because these nanostructures can be used to efficiently deliver immunological active components at specific sites (Tupin, 2013). The successful application of NT in immunology promotes the development of a new generation of vaccines and immunomodulatory medicine that can improve clinical results when treating infectious and non-infectious diseases (Smith et al., 2013). An extensive research was carried out to develop diagnostic systems with NPs. The aggregation of NPs with atypical cells inside an organism allows the early detection of pathogens and diseases through high resolution images (Sheikh et al., 2016).

#### APPLICATIONS OF NANOTECHNOLOGY IN PIG AND POULTRY INDUSTRY

Microelements like Selenium (Se) and Zn are essential for animals and are important additives that should be included in animal fodder because they prevent diarrhea and mortality of weaning pigs, as well as promote animal growth during the fattening period (Calvo et al., 2017). The weaning period is critical in piglets because of their immature physiology and immunology. These cause an increased susceptibility to infections and diseases that result in diarrhea and reduced growth rates. Consequently, the use of additives in foodstuff has become a common practice to modulate the immune system and intestinal microbes, thus promoting health, growth and yield of pigs during the postweaning period. Related to this, Milani et al. (2017) showed that ZnONPs improved the diets digestibility, increased the daily weight gain and also prevented diarrhea.

Zn due to its ability to improve intestinal function and morphology prevented diarrhea after weaning and thus promoted animal growth and increased the antioxidant capacity of weaning piglets (Zhu et al., 2017). In an analogous way, Wang et al. (2017) evaluated the effect of 1200 mg/kg of ZnONPs to promote growth in weaning pigs. Results indicated that ZnONPs ( $\pm$  30 nm) enhanced the daily weight gain and the final weight of the animals. ZnONPs significantly increased Zn concentration in blood plasma, liver and bones; and the intestinal morphology was favored as they observed an increase in villi longitude. These results suggest that ZnONPs could be dietetic for weaning piglets.

Hydrogen sulfid1e (H<sub>2</sub>S) is a toxic and corrosive gas produced as part of diverse processes in the petrol and chemical industry, as well as in livestock production, mainly in pig production. This happens because decomposition bacterial of sulphuring aminoacids in animal excreta results in the production of H<sub>2</sub>S, which afterwards is released to the atmosphere during manure management. This is alarming due to its adverse effects on human and animal health. Certain NPs can eliminate or reduce this gas concentration. Under laboratory conditions, it was shown that ZnONPs mixed with pig manure eliminate hydrogen sulfide emitted by this animal waste (Awume et al., 2017).

Scientists are working on the use of NPs to assess their functions as additives in pigs and broiler nutrition (Fondevila et al., 2009). Some of the NPs such as nanoAg, nanoSe and nanoZnO are investigated as alternatives to replace antibiotics commonly used as growth promoters in broiler and other animals of economic interest, and results have been encouraging. In a 35-day lasting study, Ali et al (2017) supplemented 40 and 80 mg ZnONPs per kg offodder and the effects on the microscopic structure of the small intestine of broiler chickens were studied. There was an increase on the growth of villi in all parts of the small intestine. Length, width, area and total number of lymph nodes in the caecal amygdala were greater in animals that were fed with fodder containing NPs.

Chromium (Cr) is an essential trace element for humans and animals. It is a component of the glucose tolerance factor; it is essential in the metabolism of carbohydrates, fats and proteins, and it likely increases the action of insulin (Eastmond et al., 2008). In pig nutrition, Cr promotes animal growth, improves meat quality and is important in reproduction. Li et al. (2017) showed that supplementing nano-chromium  $(200 \ \mu g/kg)$  to pigs with a weight higher than 76 kg increased food consumption and their average daily weight gain. Pigs had a higher Cr concentration in their blood serum and muscles, as well as less thick back fat. These authors suggested that nano-Cr increases its bioavailability and absorption capacity.

Selenium is another essential trace element for animals and has many applications in animal production. A moderate addition of Se to diet can increase the antioxidant capacity, regulate the immunological function, and promote animal growth. Gulyás et al (2017) showed that nano-Se added to broiler diets had better bioavailability and reduced toxicity compared to other Se forms used. The application rate of nanonutrients is much higher than that of micrometric size elements. Research has shown that the utilization coefficient of inorganic trace elements is around 30%, while the use coefficient of nanoelements is almost 100% (Huang et al., 2015).

Supplementing Se to chicken has been a regular practice to improve their antioxidant defense systems, as well as to increase growth; however, it has its limitations. Nano-selenium can be a better alternative as a food additive due to its higher bioavailability, wider safety margin and less toxicity, promoting in this way a better meat quality (Visha et al., 2017). The excessive cell damage resulting from oxidation could be a reason for meat quality losses in broiler. Nano-Se can be used to improve the activity of antioxidant enzymes. Chicken supplemented with 0.1875 mg/ kg nano-Se showed a significant increase in activity of superoxide dismutase and glutathione peroxidase. It seems that nano-Se improves the activity of antioxidant enzymes and promotes a higher resistance to oxidation in broiler chicken (Aparna and Karunakaran, 2016).

## NANOTECHNOLOGY IN ANIMAL REPRODUCTION

Some NPs have shown to improve fertility and to protect animal spermatozoa. Artificial insemination is important in animal reproduction. It ensures a higher number of weaning calves. NT can help to achieve this by integrating nano-techniques like non-invasive bio imaging of gametes, nanopurification and by developing protectors for cryopreservation, in order to optimize and eliminate reproductive obstacles. NPs have been explored as a method to better understand the behavior of spermatozoa and mammalian oocytes. Some inorganic NPs are of interest for reproduction because they are biocompatible, photo-stable and have a higher signal strength than fluorescent organic molecules, which are traditionally used in microscopy techniques (Bhat, 2022).

Cryopreservation of spermatozoa can be improved by adding nano-protectors, which work like buffers. They can provide the necessary nutrients for an extended storage of spermatozoa. They protect them and can be used to release antibiotics, preventing any bacterial growth that affects sperm quality and infects inseminated females (Bryla et al., 2015). Nano-sensors are studied to help understand the causes of animal abortion. On the other hand, nano-antioxidants like Zn are being used to avoid placental retention and other reproductive problems after giving birth, as well as to fight infertility problems. Zn has antioxidant properties and plays an important role in purifying reactive oxygen species (ROS). The lack of Zn can cause an increase in oxidative damage, which can contribute to poor quality of spermatozoa (Swain et al., 2016).

#### CONCLUSIONS

The potential uses of NT in veterinary medicine and animal production are huge. Due to the urgent need to increase food production for the fast growing world population, NT opens exceptional opportunities. For example, the necessary minerals in animal diets can be more efficient by using NPs that can improve flavor, palatability, color and texture; and increase nutrients bioavailability. In veterinary medicine, it can improve recovery efficiency through controlled drug release. In addition, NT has the potential to enhance artificial insemination by using some non-invasive nano techniques. However, the risk: benefit ratio that this new science implies should be taken into account, to determine if it can be used to improve the living standards of human beings.

#### REFERENCES

Ali, S., Masood, S., Zaneb, H, Faseeh-ur-Rehman, H., Masood, S, Ur Rehman, H. (2017). Pak Vet J. 2017;37(3):335-339.

Al-Sultan, S.I., Hereba, A.R.T., Hassanein, K. M., Abd-Allah, S. M., Mahmoud, U.T. and Abdel-Raheem, S.M. (2022). Italian Journal of Animal Science, 21(1), 967-978.)

Aparna, N. and Karunakaran, R. Indian J Sci Technol. 2016;9(1):1-5.

Arun, J., Sasipraba, T., Gopinath, K. P., Priyadharsini, P., Nachiappan, S., Nirmala, N. and Pugazhendhi, A. (2022). Fuel, 327, 125112.

Bhat, I.A. (2022). Reviews in Aquaculture. https://doi.org/10.1016/B978-0-12-822265-2.00009-0

Bryla, M, Trzcinska, M. (2015). Anim Reprod Sci.; 163:157-63.

Calderón-Nieva, D, Goonewardene, KB, Gomis, S, Foldvari, M. (2017). Drug Deliv Transl Res.;7(4):558-570.

Chavada, P.J. (2016). Int J Agr Sci.;8(54):2920-2922.

Coppo, J.A. (2009). Rev Vet. 20:1-61.

Eastmond, D.A., MacGregor, J.T., Slesinski, R.S. Crit Rev Toxicol. (2008). 38(3):173-190.

El Basuini, M.F., El-Hais, A.M., Dawood, M., Abou-Zeid, A.S., EL-Damrawy, S.Z., Khalafalla, M.S., Dossou, S. (2017). Aquac Nutr.;1:1-12.

Elkloub, K., Moustafa, M.E., Ghazalah, A.A., Rehan, A.A. (2015). Int J Poult. Sci.; 14(3):177-182.

Essa, S.S., El-Saied, E.M., El-Tawil, O.S., Mahmoud, M.B., El-Rahman, S.S.A. (2017). Toxicon.;140:94-104.

Elgendy, A., El-Zontahy, W. (2022). International Journal of Instructional Technology and Educational Studies, 3(3), 11-19.

Farzinpour, A, Karashi, N. (2013). In Advanced Nanomaterials and Nanotechnology Springer, Berlin, Heidelberg: 311-319.

Fondevila, M., Herrera, R., Casallas, M.C., Abecia L., Ducha, J.J. (2009). Anim Feed Sci Technol. 150(3):259-269.

- Gahlawat, G., Shikha, S., Chaddha B.S., Chaudhuri, S.R., Mayilraj, S., Choudhury, A.R. (2016). Microb Cell Fact.;15(1):1-14.
- Gangadoo, S., Stanley, D., Hughes, R.J., Moore, R.J., Chapman, J. (2016). Trends Food Sci Technol.; 58:115-126.
- Ganjigohari S, Ziaei N, Ramzani GA, Tasharrofi S. (2017). J Anim Physiol Anim Nutr. 102:225-232.
- Goel, A., Bhanja, SK., Mehra, M., Majumdar, S., Mandal, A. (2017). Arch Anim Nutr.;71(5):384-394.
- Gonzales-Eguia, A., Fu, C.M., Lu, F.Y., Lien, T.F. (2009). Livest Sci. 2009;126(1):122-129.
- Greenwood, D.L., Dynon, K., Kalkanidis, M., Xiang, S., Plebanski, M., Scheerlinck, J.P. (2008). Vaccine Rep. 26:2706-2713.
- Hameed, H.M. (2021). Egyptian Journal of Veterinary Sciences, 52(3), 311-317.
- Hill, E.K., Li, J. (2017). J Anim Sci Biotechnol;8(1):1-13.
- Huang, S., Wang, L., Liu, L., Hou, Y., Li, L. (2015). Agron Sustain Dev. 15;35(2):369-400.
- Khah, M.M., Ahmadi, F., Amanlou, H. (2015). Indian J Anim Sci. 85(3):287-290.
- Li, T.Y., Fu, C.M., Lien, T.F. (2017). Anim Prod Sci.;57(6):1193-1200.
- Milani, N.C., Sbardella, M., Ikeda, N.Y., Arno, A., Mascarenhas, B.C., Miyada, V.S. (2017). Anim Feed Sci Technol.; 227:13-23.

Mohammadi, H., Farzinpour, A., Vaziry, A. (2011). J Appl Environ Biol Sci. 11;1(10):414-419.

- Prasad, R.D., Sahoo, A.K., Shrivastav, O.P., Charmode, N., Kamat, R., Kajave, N.G., Prasad, N.R. (2022). ES Food & Agroforestry, 8: 12-46.
- Rassouli, A., Al-Qushawi, A., Atyabi, F., Peighambari, S.M, Esfandyari-Manesh, M., Shams, G.R. (2016). Turk J Vet Anim Sci. 40(5):540-547.
- Shabani, A., Dastar, B., Hassani, S., Khomeiri M., Shabanpour, B. (2016). J Agric Sci Technol. 18(1):109-121.
- Sheikh, S.E, Hasanabadi, A., Golian, A. (2016). Iran J Appl Anim Sci.;8(1):96-107.
- Smekalova, M., Aragon V., Panacek, A., Prucek R., Zboril, R., Kvitek, L. (2016). Vet J. 209:174-179.
- Smith, D.M., Simon, J.K., Baker, Jr. J.R. (2013). Nat Rev Immunol. 13(8):592-605.
- Swain, P.S., Rajendran, D., Rao, S.B.N, Domonic G. (2015). Vet World. 8(7):888-891.
- Swain, P.S., Rao, S.B., Rajendran, D., Dominic, G., Selvaraju, S. (2016). Anim Nutr. 2(3):134-141.
- Thulasi, A., Rajendran, D., Jash, S., Selvaraju, S., Jose, V.L., Velusamy, S., Mathivanan, S. (2013). Anim Nutr Reprod Phys. Chapter 24, Pp. 500-515.
- Tupin, E. (2013). 9:9, 2009-2012, DOI: 10.4161/hv.25934.
- Visha, P., Nanjappan, K., Selvaraj, P., Jayachandran, S., Thavasiappan, V. (2017). Int J Curr Microbiol App Sci.;6(5):340-347.
- Wang, C., Zhang, L., Su, W., Ying, Z., He, J., Zhang, L., Wang, T. (2017). PloS one. 12(7):1-13.

- Xing, N., Guan, X., An, B., Cui, B., Wang, Z., Wang, X., Tong, D. (2016). PloS one. 11(12):1-16.
- Yang, T., Gan, Y.N., Song, Z.F., Zhao, T.T, Gong, YS. (2014). Chin J Anim Nutr. 3:659-666.
- Zhao, Y., Feng, Y.N., Li, L., Zhang, H.F., Zhang, Y.N., Zhang, P.F., Shen, W. (2017). Biol Trace Elem Res. 177(2):353-366.
- Zhu, C., Lv, H., Chen, Z., Wang, L., Wu, X., Chen, Z., Jiang, Z. (2017). Biol Trace Elem Res. 175(2):331-338.

Zinsstag, J., Schelling, E., Waltner-Toews, D, Tanner, M. (2011). Prev Vet Med. 101(3):148-156.