International Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

IJBSM May 2026, 17(5): 01-06

Article AR6945

Research Article

Natural Resource Management

DOI: HTTPS://DOI.ORG/10.23910/1.2026.6945

Selenium Nanoparticles Synthesized by Ball Milling Method: Their Characterization to Incorporate in Poultry Feed

Arun Bharathi[1], S. Ezhil Valavan[2], P. Veeramani[3], M. Ananda Chitra[4], J. Ramesh[5] and R. Richard Churchill[6]

[1]Dept. of Livestock Farm Complex, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry (605 009), India
[2]Pharmacovigilance Laboratory for Animal Feed and Food Safety, [4]Translational Research Platform for Veterinary Biologicals, [6]Poultry Research Station, Madhavaram Milk Colony, Chennai, Tamil Nadu (600 051), India
[3]Dept. of Poultry Science, [5]Dept. of Animal Nutrition, Madras Veterinary college, Chennai, Tamil Nadu (600 007), India

Open Access

Corresponding arunbharathinithya@gmail.com

0009-0004-9059-6430

ABSTRACT

The experiment was conducted in April, 2023 at Madras Veterinary College, Chennai, India to study the efficiency of nanoselenium in alleviating heat stress in broilers. To incorporate nana Se in poultry diet, the selenium nanoparticles were synthesized by the ball milling technique (physical method) using feed-grade sodium selenite powder at 250 rpm in a planetary ball mill. The result showed that the product yield of synthesized nano selenium as 95.04% after milling for five hour thirty minutes. The dynamic light scattering technique was used to analyse the particle size and zeta potential of nano Se, which resulted in 69.1 nm and –23.9, respectively. The X-ray Diffraction revealed that the selenium nanoparticle was crystalline in nature. The Fourier Transform Infra-Red Spectroscopy analysis displayed the characteristic peaks at 3437, 2923 and 2851, 1601, 1427 corresponding to the presence of O-H, C-H, C=O, COO- functional groups, respectively which show the stability of synthesized nano selenium. The well-defined peak in the FTIR spectrum confirmed that there were no impurities in the synthesized nano Se. The transmission electron microscope unveiled the size of nano Se in the range of 6–20 nm and the shape of synthesized nano Se was spherical in nature. Hence, it can be concluded that the nano selenium synthesized productively through a physical method and can be utilized as a mineral supplement in the commercial poultry diet.

KEYWORDS: FTIR, nano selenium, TEM, X-ray diffraction, zeta potential

***Citation* (VANCOUVER):** Bharathi et al., Selenium Nanoparticles Synthesized by Ball Milling Method: Their Characterization to Incorporate in Poultry Feed. *International Journal of Bio-resource and Stress Management,* 2026; 17(5), 01-06. HTTPS://DOI.ORG/10.23910/1.2026.6945.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, author have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The author has declared that no conflict of interest exists.

RECEIVED on 23rd January 2026 RECEIVED in revised form on 22nd April 2026 ACCEPTED in final form on 09th May 2026 PUBLISHED on 16th May 2026

1. INTRODUCTION

Minerals are essential for growth, egg production and reproduction. Trace minerals could also be used effectively to alleviate some of the adverse effects of the heat stress. Minerals like Zn and Se are the most important minerals used in the antioxidative defense system in the heat-stressed poultry (Mir et al., 2018). Selenium (Se) is one of the indispensable minerals required for better growth performance and production in poultry, as it is a cofactor for many antioxidant enzymes. Se is an integral part of more than 25 selenoproteins, of which glutathione peroxidases (GPx) and thioredoxin reductases (TrxR) have roles in detoxification, maintenance of the redox potential and antioxidant defense enzyme systems control free radicles of reactive oxygen species and Se involves in all aspects of immune function (Nabi et al., 2020). Se increases the Cu-Zn-superoxide dismutase and glutathione peroxide activity, protects the mucosa of the small intestine and pancreas from oxidative damage during heat stress in birds (Placha et al., 2014). Se has a vitamin E-sparing role by enhancing its absorption from the gut and protecting the cell membrane fats from oxidative damage (Habibian et al., 2015). The recommended concentration of Se in commercial broiler chicken throughout its growing period is 0.15 mg kg^{-1} of diet (Anonymous, 2007). Se deficiency in chickens promotes gut inflammation (Gao et al., 2016; Wu et al., 2016) and also the metabolism of minerals is negatively impacted by heat stress. Heat stress causes the body to lose too many minerals, which not only makes it harder for chickens to adapt but also increases the risk of chronic diseases. When birds are under heat stress, their appetites are lowered, which leads to a decrease in the amount of minerals they consume (Sahin et al., 2009). Sahoo et al. (2014) reported that nanoparticles are likely to be distinct from their normal forms due to their extremely small size and unique physical features. These are intended to have greater absorption, a low dose rate and a steady interaction with other components as a feed supplement. Gopi et al. (2017) opined that the major application of nanotechnology in poultry involves use of nano mineral elements, which can reduce any antagonistic behavior seen in traditional inorganic minerals in the gastrointestinal tract and improve the bioavailability at lower effective doses. Moreover, higher bioavailability and better utilization of trace minerals could have a desirable effect on metabolism, while reducing mineral excretion into the environment (Surai et al., 2017). The nano Se was produced by grinding feed grade sodium selenite in ball mill @ 1060 cycles per minute using two 12.7 mm stainless steel balls in 75 ml capacity jar for 60 mins (High energy ball milling technique (Physical method) with product yield of 50 gram per hour (Jamima et al., 2020). The main parameters in the characterization of nanoparticles are size and shape of the particle. In addition, the crystal structure and the chemical composition should be thoroughly investigated as first part of the nanoparticle synthesis (Mourdikoudis et al., 2018). The methods used to characterize nanoparticles are dynamic light scattering (particle size), zeta potential (stability), X-ray diffraction (microstructure), Transmission Electron Microscope (morphology) and Fourier Transform Infra-Red Spectroscopy (functional groups) (Kazemi et al., 2021). Therefore, the nano form of minerals can be introduced into poultry feed to increase absorption and efficient use of minerals during stress. This study was conducted to synthesize and characterize the nano selenium by physical methods to incorporate it in poultry feed.

2. MATERIALS AND METHODS

The experiment was conducted in April of 2023 at Madras Veterinary College to study efficiency of nanoselenium in alleviating heat stress in broilers. The ball milling technique (physical method) was used to synthesize nano Se using a planetary ball mill (Retsch, 100). The process was standardized with different milling times, the number of zirconium balls, and quantities of sodium selenite used. Finally, a nano form of Se was synthesized by grinding 4 grams of feed-grade sodium selenite in a 50 ml capacity zirconium jar with 40 zirconium balls of 5 mm diameter for 5 hours and 30 minutes at 250 cycles $minute^{-1}$.

Nano particle product yield (%) was determined by dividing the nano material obtained after grinding by the quantity of mineral source used for grinding in the ball mill, multiplied by 100.

Nano particle yield (%)=(Nano material obtained after grinding/ Quantity of raw material used)×100

The synthesized nano Se was characterized with the following techniques (Kazemi et al., 2019): particle size analysis and transmission electron microscope technique for analysis of particle size, zeta potential to analyze the stability, X-ray diffraction technique using Rigaku-Mini Flex-II Desktop X-ray Diffractometer (Theivasanthi and Alagar, 2010) to unveil the crystallinity and particle size of the crystal, Fourier Transform Infra-Red Spectroscopy (Chattopadhyay et al., 2014) to reveal surface chemistry and functional group of synthesized nanoparticles.

The average particle size of nano Se was derived through XRD by using the Debye-Scherrer formula (Mustapha et al., 2019)

$D=0.9\lambda/\beta \cos \theta$

Where 'λ' is wave length of X-Ray (0.1541 nm), 'β' is the FWHM (full width at half maximum), 'θ' is the diffraction angle, and 'D' is the particle diameter.

3. RESULTS AND DISCUSSION

The synthesized nano Se by the physical method was weighed, and the product yield of synthesized nano Se derived using the formula was 95.04%. The particle size of synthesized nano Se by the ball milling technique under a standardized procedure was below 100 nm as resolved by particle size analyzer, XRD, and TEM techniques (Table 1).

Table 1: Characterization of synthesized nano Selenium

Sl. No.	Characterization parameters	
1.	Chemical source	Sodium selenite
2.	Mean product yield (%)	95.04
3.	Particle size (assessed through particle size analyzer)	69.1 nm
4.	Particle size (assessed through TEM)	6-20 nm
5.	Particle size (Debye-Scherrer formula)	43.26 nm
6.	Zeta potential (mV)	-23.9
7.	XRD	Free of impurities
8.	FTIR	Stability of nanoparticles

3.1. Particle size and zeta potential

The particle size distribution and zeta potential of the synthesized Se are depicted in Figure 1. The particle size of the synthesized nano-Se was assessed by the dynamic light scattering (DLS) technique as 69.1 nm. Nano Se with particle size range from 10–80 nm and 74–76 nm, 137.85 nm has been recorded in DLS by Pardechi et al. (2020) and Arulnathan et al. (2016), Sun et al. (2023) respectively, which is in agreement with the result of the present study. The zeta potential of synthesized nano-Se is depicted in Figure 2. The zeta potential is used to study the stability of the synthesized Se nano particles as -23.9. Similar findings were reported by Arulnathan et al. (2016)

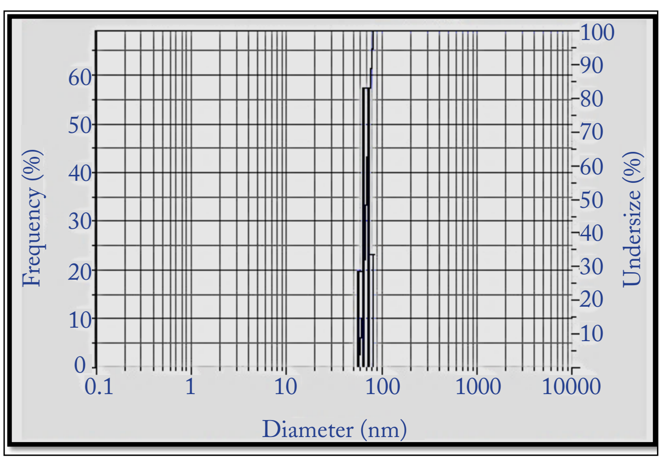

Figure 1: Particle size distribution of synthesized nano Se in particle size analyzer

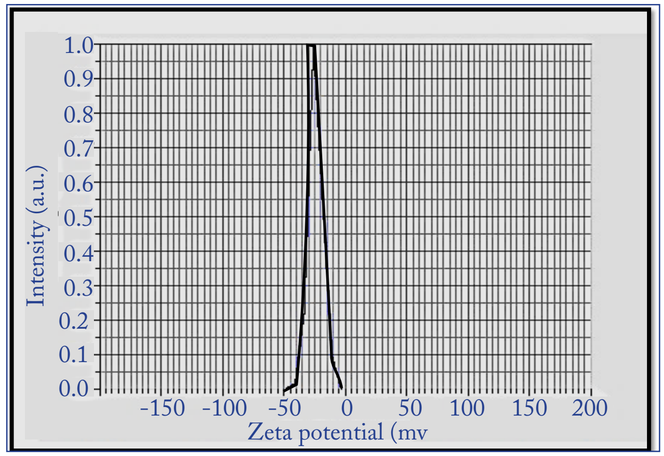

Figure 2: Zeta potential of synthesized nano Se

and Jamima et al. (2020), the zeta potential of nano Se was -22.8 and -23.30 mV, respectively. The zeta potential of +30 to -30 mV is considered to have a high degree of stability in anionic and cationic nano-particles, respectively (Xu, 2012, and Clogston and Patri, 2011), which indicates that the synthesized nano Se is highly stable in nature.

3.2. X-ray diffraction

XRD yields the information on crystalline structure, phase, and crystalline grain size (Mourdikoudis et al., 2018). The XRD pattern of the synthesized Se nanoparticles is depicted in Figure 3. The sharp diffraction peaks in the nano Se graphs indicated that the synthesized Se nanoparticles have high crystallinity. The XRD peaks proved that the produced powder of Se is pure because no other characteristic XRD peaks were detected other than the nano Se. A similar finding was reported by Jamima et al. (2020). The particle size of the synthesized nanoparticles was calculated using the Debye-Scherrer formula as approximately 43.26 nm. XRD can be utilized to yield information on crystallinity, particle size, and shape of nanoparticles. In our study, the XRD peak indicates that the synthesized nano-Se is highly crystalline and free of impurities.

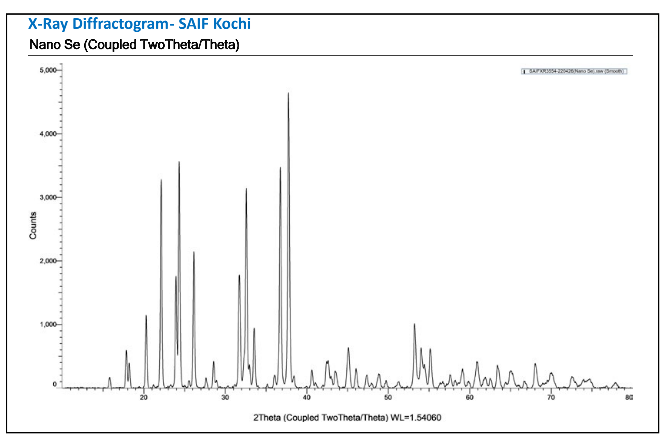

Figure 3: Powder XRD patterns of synthesized Se powder

3.3. *Fourier transform infra-red spectroscopy*

The functional group of synthesized nano-Se was identified by the FTIR spectroscopy technique, which also unveils the chemical bonding of the nanoparticle (Ramiah et al., 2019, and Baudot et al., 2010). The FTIR spectrum of the synthesized nano-Se is depicted in Figure 4. The peaks indicate that the characteristic functional group is present in the synthesized Se nanoparticles. FTIR spectrum for synthesized nano Se was located at 3437.18, 2923.48, 2851.85, 1601.38, 1519.50, 1427.40, 1404.89, 788.62, 738.78, and 452.70 cm^{-1}. The observed FTIR results confirmed that the synthesized Se nanoparticle was without any significant impurities. Similar to the finding of Jamima et al. (2020), it was observed that the shift in peak from 3437.18 to 2851.85 cm^{-1} corresponded to OH/C-C bending, which might be due to the equilibrium of nano Se.

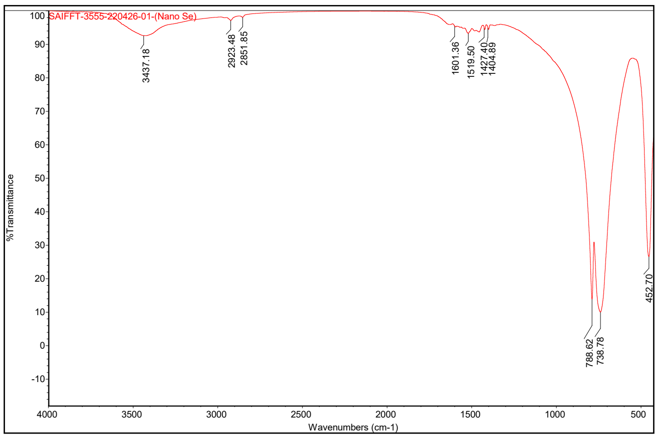

Figure 4: FTIR spectra of synthesized nano-Se

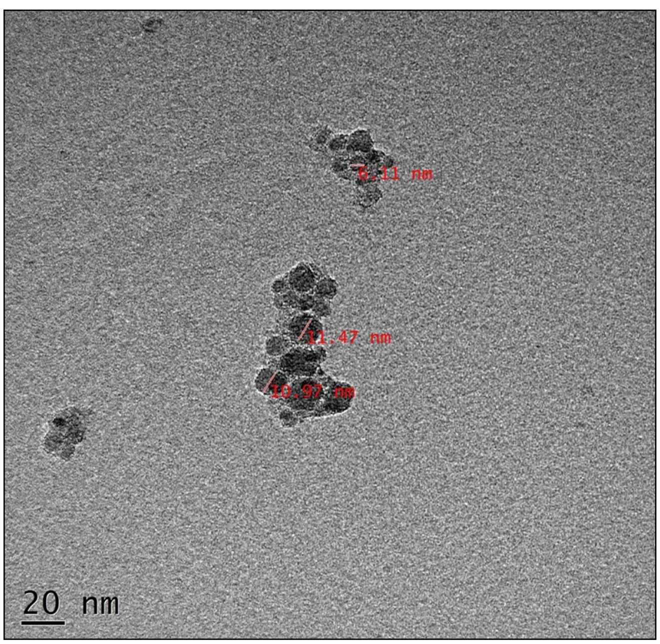

Figure 5: TEM micrographs of synthesized nano-Se

3.4. *Transmission electron microscopy*

The TEM of the nanoparticle source of Se is depicted in Figure 5. The size of synthesized Se nanoparticles was analysed by TEM, which was in the range of 6-20 nm. The mean size assessed through TEM was 9.52 nm for the synthesized nanoforms of Se. Similarly, Wang et al. (2019) mentioned that TEM is a useful method to analyse the size, form, and homogeneity of nanoparticles. Similar to the findings of Arulnathan et al. (2016), Alam et al. (2019), and Pardechi et al. (2020), TEM revealed the shape of nano Se synthesized in this study as spherical.

4. CONCLUSION

The ball milling technique (Physical method) was used productively to synthesize the nano Se from the sodium selenite. The characterization by particle size analysis, zeta potential, XRD, FTIR and TEM revealed that the synthesized nano Se were met all the requisites characteristics of nanoparticle. The synthesized nano-Se can be used as a feed supplement in the poultry diet as nano Se bioavailability better than that of other forms of Se.

5. ACKNOWLEDGEMENT

I would like to acknowledge DST SAIF, Sophisticated Test and Instrumentation Centre (STIC), Cochin University of Science & Technology Campus, Kochi, for providing the facilities to carry out the techniques used in this study.

6. REFERENCE

Alam, H., Khatoon, N., Raza, M., Ghosh, P.C., Sardar, M., 2019. Synthesis and characterization of nano selenium using plant biomolecules and their potential applications. BioNanoScience 9(1), 96-104. https://doi.org/10.1007/s1266801805695?urlappend=%3Futm_source%3Dresearchgate.net%26utm_medium%3Darticle.

Arulnathan, N., Karunakaran, R., Balakrishanan, V., Chellapandian, M., Geetha, K., 2016. Synthesis and characterization of nano selenium as a feed supplement. International Journal of Environmental Science and Technology 5(4), 2296–2300. https://www.researchgate.net/publication/346522271_.

Baudot, C., Tan, C.M., Kong, J.C., 2010. FTIR spectroscopy as a tool for nano-material characterization. Infrared Physics and Technology 53(6), 434–438. https://doi.org/10.1016/j.infrared.2010.09.002.

Anonymous, 2007. BIS, 2007. Indian Standard Poultry Feeds – Specification. 5th Edn. New Delhi. Available at: https://law.resource.org/pub/in/bis/S06/is.1374.2007.pdf. Accessed Date: 17th February, 2023 .

Chattopadhyay, S., Dash, S.K., Tripathy, S., Das, B., Mandal, D., Pramanik, P., Roy, S., 2014. Toxicity of cobalt oxide nanoparticles to normal cells: an in vitro and in vivo study. Chemico-Biological Interactions 226, 58-71. https://doi.org/10.1016/j.cbi.2014.11.016

Clogston, J.D., Patri, A.K., 2011. Zeta potential measurement. In the characterization of nanoparticles intended for drug delivery. Humana Press. 63–70p. https://doi.org/10.1007/978-1-60327-198-1_6.

Dlouha, G., Sevcikova, S., Dokoupilova, A., Zita, L., Heindl, J., Skrivan, M., 2008. Effect of dietary selenium sources on growth performance, breast muscle selenium, glutathione peroxidase activity, and oxidative stability in broilers. Czech Journal of Animal Science 53, 265–269. DOI: 10.17221/361-CJAS.

Gao, X., Zhang, Z., Xing, H., Yu, J., Zhang, N., Xu, S., 2016. Selenium deficiency induced inflammation and increased expression of regulating inflammatory cytokines in the chicken gastrointestinal tract. Biological Trace Element Research 173, 210–218. . https://doi.org/10.1007/s12011-016-0651-1.

Gopi, S.P., Balakrishnan, A., Pius, S., Thomas, 2017. Chitin nano whisker (ChNW)-functionalized electrospun PVDF membrane for enhanced removal of Indigo carmine. Carbohydrate Polymers 165, 115–122. https://doi.org/10.1016/j.carbpol.2017.02.046.

Habibian, M., Sadeghi, G., Ghazi, S., Moeini, M.M., 2015. Selenium as a feed supplement for heat-stressed poultry: a review. Biological Trace Element Research 165, 183–93. Available from: https://pubmed.ncbi.nlm.nih.gov/25721721/.

Jamima, J., Veeramani, P., Kanagaraju, P., Kumanan, K., 2020. Synthesis and characterization of selenium nanoparticles by High Energy Ball Milling (HEBM). Indian Journal of Veterinary and Animal Science Research 49(4), 45–51. https://doi.org/10.56093/ijvasr.v49i4.130865.

Kazemi, M.A., Akbari, S., Soleimanpour, N., Feizi, M., Darroudi, 2019. The role of green reducing agents in gelatin-based synthesis of colloidal selenium nanoparticles and investigation of their antimycobacterial and photocatalytic properties. Journal of Cluster Science 30(3), 767–775. https://doi.org/10.1007/s10876-019-01537-4.

Kazemi, S., Pourmadadi, M., Yazdian, F., Ghadami, A., 2021. The synthesis and characterization of targeted delivery curcumin using chitosan-magnetite-reduced graphene oxide as nano-carrier. International Journal of Biological Macromolecules 186, 554–562. https://doi.org/10.1016/j.ijbiomac.2021.06.184.

Mir, S.H., Pal, R.P., Mani, V., Malik, T.A., Ojha, L., Yadav, S., 2018. Role of dietary minerals in heat-stressed poultry: A review. Journal of Entomology and Zoology 6(5), 820–826. Available from: https://www.researchgate.net/profile/Shahid-Mir/publication/327980644.

Mustapha, S., Ndamitso, M.M., Abdulkareem, A.S., Tijani, J.O., Shuaib, D.T., Mohammed, A.K., Sumaila, A., 2019. Comparative study of crystallite size using Williamson-Hall and Debye-Scherrer plots for ZnO nanoparticles. Advances in Natural Sciences: Nanoscience and Nanotechnology 10(4), 045013. 10.1088/2043-6254/ab52f7.

Mourdikoudis, S., Pallares, R.M., Thanh, N.T., 2018. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. Nanoscale 10(27), 12871–12934. https://doi.org/10.1039/C8NR02278J.

Nabi, F., Arain, M.A., Hassan, F., Umar, M., Rajput, N., Alagawany, M., Liu, J., 2020. Nutraceutical role of selenium nanoparticles in poultry nutrition: a review. World's Poultry Science Journal 76(3), 459–471. https://doi.org/10.1080/00439339.2020.1789535

Pardechi, A., Tabeidian, S.A., Habibian, M., 2020. Comparative assessment of sodium selenite, selenised yeast, and nanosized elemental selenium on performance response, immunity, and antioxidative function of broiler chickens. Italian Journal of Animal Science 19(1), 1108–1121. https://doi.org/10.1080/1828051X.2020.1819896.

Placha, I., Takacova, J., Ryzner, M., Cobanova, K., Laukova, A., Strompfova, V., 2014. Effect of thyme essential oil and selenium on intestinal integrity and antioxidant status of broilers. British Poultry Science 55, 105–114. https://doi.org/10.1080/00071668.2013.873772.

Ramaiah, K., Srishailam, K., Reddy, K.L., Reddy, B.V., Rao, G.R., 2019. Synthesis, crystal and molecular structure, and characterization of 2-((2-aminopyridin-3-yl) methylene)-N-ethylhydrazinecarbothioamide using spectroscopic (1H and 13C NMR, FT-IR, FT-Raman, UV–Vis) and DFT methods, and evaluation of its anticancer activity. Journal of Molecular Structure 1184, 405–417. https://doi.org/10.1016/j.molstruc.2023.136339.

Sahin, K., Sahin, N., Kucuk, O., Hayirli, A., Prasad, A.S., 2009. Role of dietary zinc in heat-stressed poultry: A review. Poultry Science 88, 2176–2183. https://doi.org/10.3382/ps.2008-00560.

Sahoo, A.R.K., Swain, S.K., Mishra, B., Jena, 2014. Serum biochemical indices of broiler birds fed on inorganic, organic, and nano zinc-supplemented diets. International Journal of Recent Scientific Research 5(11), 2078–2081. https://www.researchgate.net/publication/304497057_.

Sun, J., Li, J., Yao, L., You, F., Yuan, J., Wang, D., Gu, S., 2023.

Synthesis, characterization, and antioxidant activity of selenium nanoparticle decorated with polysaccharide from hawthorn. Journal of Food Measurement and Characterization 17(6), 6125–6134. Available from: https://doi.org/10.1007/s11694-023-02124-y?urlappend=%3Futm_source%3Dresearchgate.net%26utm_medium%3Darticle.

Surai, P.F.T.T., Kochish, O.A., Velichko, 2017. Nano-Se assimilation and action in poultry and other monogastric animals: Is gut microbiota an answer? Nanoscale Research Letters 12(1), 1–7. https://doi.org/10.1186/s11671-017-2383-3.

Theivasanthi, T., Alagar, M., 2010. X-ray diffraction studies of copper nanopowder. Archives of Physics Research. 1(2), 112–117. Available from: https://www.researchgate.net/publication/45909455_X.

Wang, Y., Peng, X., Abelson, A., Zhang, B.K., Qian, C., Ercius, P., Zheng, H., 2019. *In situ* TEM observation of neck formation during oriented attachment of PbSe nanocrystals. Nano Research 12, 2549–2553. https://doi.org/10.1007/s12274-019-2483-8.

Wu, C., Xu, Z., Huang, K., 2016. Effects of dietary selenium on inflammation and hydrogen sulfide in the gastrointestinal tract in chickens. Biological Trace Element Research 174, 428–435. https://doi.org/10.1007/s12011-016-0735-y.

Xu, M.J., Li, H., Iwai, Q., Mei, D., Fujita, H., Su, H., Chen, N., Hanagata, 2012. Formation of nano-bio-complex as nanomaterials dispersed in a biological solution for understanding nanobiological interactions. Scientific Reports 2(1), 1–6. https://doi.org/10.1038/srep00406.