

Effect of Nano Zinc Oxide on Some Behaviour Patterns and Performance in Calves

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Abstract:

This study was conducted to evaluate the effect of Nano zinc oxide particles (nZnO) supplementation on growth performance, ingestive and standing idle behaviours, serum zinc level and some haematological parameters in cross bred calves. Ten calves at the farm of Faculty of Veterinary Medicine – Aswan University were randomly selected within an average age of 4 months and weight of (70.3±5.3 kg). Calves were divided equally into two groups, control, and treated groups (n=5) in each group. Each treated calf was administered daily with zinc oxide Nano particles at the rate of (10µg/g) orally for three months. The results revealed that the used rate of nZnO supplementation had no significant effect on the average daily gain of calves throughout the different periods of experiment. Moreover, the average time spent in ingestive behaviour was significantly higher in control group (18.1±0.3 and 16.8±1.4 min) than treated group (11±2.1 and 5.7±3 min) after 2 weeks and 8 weeks respectively. On the other hand, serum zinc level was significantly higher (p<0.05) in the treated group (9.2±0.4 µg/dl) than control group (8.0±0.2 µg/dl) after the 12th week. There was no significant effect of nZnO supplementation on the other measured hematological parameters. In conclusion, nZnO supplementation may have a little effect on ingestive and standing idle behaviour but it has no pronounced effect on growth performance, serum zinc level and some haematological parameters in calves.

Keywords: Nano zinc, Calves, Behaviour, Growth performance, Hematological parameters.

1- Introduction

Calves play an important role in the animal production enterprises and farm economics. They represent the major source of herd replacement, and the beef production stocks. So, they greatly share in solving the problem of milk and beef shortage in the developing countries which will be reflected on the welfare of human being.

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Nowadays under modern intensive methods of calf management it become necessary to improve management practices to reduce suboptimal performance and mortalities and to fulfill calf welfare. (Djemali ,2020 ; Radchikov et al., 2021). Rearing calves using modern technology, feed and feed additives is critical for a successful dairy or beef cattle breeding. Maximum productivity at the lowest cost can be obtained from healthy calves only. In this regard, it is necessary to constantly improve the calves feeding system and develop new feed additives (Marques et al., 2019).

Zinc, as a trace mineral caters to several needs of the animal directly or indirectly which support the functions like growth, skin health, hair and hoof growth, wound healing, cell division and reproduction. The performance of growing animals is severely affected even upon a minute deficiency of trace minerals and zinc is not an exception. (Anil et al., 2019)

Nano engineered product, the Nano zinc oxide (nZnO), a new form of zinc metal has been designed by using the principles of nanotechnology where the size, shape and crystalline structure of the metal are manipulated by various methods of preparation (Swain et al., 2016).

Few workers across the world investigated dietary nZnO supplementation on growth performance in poultry and small ruminants. Many of the work reported a positive conclusion that inclusion of nZnO for conventional zinc showed an improvement in the feed intake (Ahmadi et al., 2013), growth rates (Mishra et al., 2014) and favorable FCR (Zhao et al., 2014 ; Sahoo et al., 2016) in various species. There is a lack of information on the literature investigating the effect of trace elements on behaviour of calves. There is a paucity of data describing the effect of Nano zinc oxide on behaviour of calves. Mousavi – Haghshenas et al. (2022) found a significant effect of dietary mineral source on different behaviours in calves. Prior studies declared that addition of nZnO has no clear effect on the hematological parameters in calves (Feldman et al., 2002; Nagalakshmi et al., 2015; Swain et al., 2019).

The objective of this study aimed to evaluate the effect of nZnO on growth performance, ingestive and standing idle behaviours, serum zinc level and some hematological parameters in calves.

2- Materials and Methods

1- Animal management:

This study was carried out on ten calves at the farm of Faculty of Veterinary Medicine – Aswan University. Calves were randomly selected with an average age of 4 months and weight of 70.3 ± 5.3 kg

Calves were tied in a partially sheltered yard with a separate manager for each one. Concentrated feed was given according to body weight(NRC,2001), while hay and straw were introduced *ad libitum*. Calves were divided equally into two groups, control and treated (5 in each group). Each calf in the treated group was administered daily with zinc oxide Nano particles(NanoTech Egypt for photo-electronics with white powder properties, a size of 30 ± 5 nm on average, and a spherical-like shape.) at the rate of 10 ppm($10 \mu\text{g/g}$) orally for three months.

2- Performance measures:

Each calf was weighed every two weeks during the experimental period by using an electronic platform weighing scale. Average daily gain was measured every two weeks (Anil et al., 2019).

3- Behavioural measures:

Ingestive and standing idle behaviours of each calf were monitored twice daily at 9 a.m. and 4 p.m. continuously for 15 minutes for each recording by using a video camera with a total observation period of 30 minutes (Altman, 1974; Martin and Bateson, 1993). The average time for ingestive and standing idle behaviors was calculated through the period of study.

4- Sampling and hematological parameters estimation:

Blood Samples were collected from the jugular vein and divided into vacutainer tubes containing EDTA for hematological examination. Including red blood cells (RBCS) count, hemoglobin concentration, and packed cell volume (PCV) while other samples were collected in vacutainer tubes without anticoagulant for biochemical investigation. Samples were left for about 15 minutes till complete clotting then centrifuged at 1500 rpm for 30 minutes for serum separation. The resultant serum then transferred by Pasteur pipette to labeled Eppendorf tubes which were kept by deep freezing at -20°C till assayed.

RBCS count ($10^{12}/l$) was performed using an improved Neubauer chamber. Blood was diluted with RBCs diluting solution, counted and calculated according to standard method described by (Jain, 1986). Hemoglobin concentration Hb (g/dl) was determined by colorimetric endpoint cyanomethaemoglobin method using Drabkin solution (Tietz, 1976). Packed cell volume (PCV%) was determined by the microhaematocrit centrifugation technique (Jain, 1986). Zinc concentration in serum ($\mu\text{g}/\text{dl}$) was measured. Zinc level was determined using colorimetric assay based on the chelation of zinc present in the sample by adding zincon (2-carboxy-2'-hydroxy-5-sulphoformazyl-benzene) in the reagent at alkaline pH. The formation of this complex was measured at a wavelength of 610 nm (Hayakawa et al., 1961).

5-Statistical analysis:

Data were analyzed statistically with a student t-test by using SAS software (SAS, 2008). Data were expressed as mean \pm standard error of mean. Values of $p < 0.05$ were considered significant

3- Results and Discussion

The effect of Nano zinc oxide particles (nZnO) on body weight of calves is shown in Table (1). The result revealed that there was no significant difference ($p > 0.05$) in body weight of calves in the control group at the start of experiment and after 2, 4, 6, 8, 10 and 12 weeks (69, 82.6, 94.4, 106.6, 118, 128 and 137.6 kg) as compared to treated group (71.6, 84.4, 99.8, 112, 122.4 130.2, and 138 kg) respectively. These results agree with early studies (Mandal, 2004; Jadhav, 2005; Nagalaskmi et al., 2013; Zaboli et al., 2013) but other researches (Ahmadi et al., 2013; Mishera et al., 2014; Sahoo et al., 2016) disagree with the obtained finding.

Table (1). Effect of Nano zinc oxide particles on body weight of calves.

Experimental period	Body weight(kg)	
	Control	Treated
Initial	69 \pm 5.3	71.6 \pm 3.7
After 2 weeks	82.6 \pm 4.1	84.8 \pm 3.3
After 4 weeks	94.4 \pm 5.5	99.8 \pm 4.2
After 6 weeks	106.6 \pm 6.5	112 \pm 4.3
After 8 weeks	118 \pm 6.8	122.4 \pm 3.6
After 10 weeks	128 \pm 6.6	130.2 \pm 3.8
After 12 weeks	137.6 \pm 6.4	138 \pm 3.7

Results expressed as mean \pm SE

Concerning the results in Table (2), it could be explained that there was no significant difference ($p>0.05$) between ADG of calves in the control and treated group throughout the period of experiment at 2, 4, 6, 8, 10 and 12 weeks. This may be attributed to the low level of administered nZnO 10ppm(10 μ g/g) in the experiment which has no clear effect on growth of calves. These findings were in harmony with **Kessler et al. (2003)** who found that the supplementation of zinc in the form of Zn oxide, Zn proteinate and Zn polysaccharide with level of 45 mg/kg DM to fattening bulls(4–6-week-old Red Holstein \times Simmental calves) had no significant impact on growth performance. Moreover, **Wright et al. (2004)** found that Zinc supplementation of Forty-eight Holstein male calves with 20 mg of Zn/kg of DM as ZnSO₄ or Zn proteinate (ZnProt) for 98 days did not have a significant impact on ADG. Furthermore, **Zaboli et al., (2013)** didn't find any effect of addition of NZno with a level of 20 or 40 ppm on ADG in Iranian angora kids with average age of 5 – 6 months fed control diet containing 22 ppm nano zinc. Our findings disagree with other studies (**Mc Dowell 1985 ;Mishera et al., 2014**).

Table (2). Effect of Nanozinc oxide particles on average daily gain of calves.

Experimental period	Average daily gain (kg/d)	
	Control	Treated
After 2 weeks	0.9 \pm 0.1	0.9 \pm 0.2
After 4 weeks	0.8 \pm 0.1	1.1 \pm 0.1
After 6 weeks	0.9 \pm 0.1	0.9 \pm 0.1
After 8 weeks	0.8 \pm 0.1	0.7 \pm 0.1
After 10 weeks	0.7 \pm 0.1	0.6 \pm 0.1
After 12 weeks	0.7 \pm 0.1	0.6 \pm 0.1

Results expressed as mean \pm SE

The average time spent in ingestive behaviour was significantly increased ($p<0.05$) in control group (18.1 \pm 0.3 and 16.8 \pm 1. 4) than treated group (11 \pm 2.1 and 5.7 \pm 3) after 2 weeks and 8 weeks respectively (Table 3). However, there was no significance difference ($p>0.05$) at the remaining periods of the experiment in this respect. The results concerning standing idle behaviour were variable throughout the different period of experiment which can not be interpreted. The effect of feed additives on behaviour of calves are not mentioned before in different literatures and there is a paucity of data describing the effect of nZnO in dairy calves (**Heisler et al., 2020**). Calves fed advanced chelated minerals spent more time eating and less time to lying down after weaning (**Mousavi-Haghshenas et al., 2022**).

Table (3). Effect of Nano zinc oxide particles on ingestive and standing idle behaviours of calves.

Experimental period	Control		Treated	
	Ingestive behaviour	Standing idle behavior	Ingestive behaviour	Standing idle behavior
Initial	8.4 \pm 1.7	20.2 \pm 1.7 ^a	11.9 \pm 1.9	9.9 \pm 2.8 ^b
After 2 weeks	18.1 \pm 0.3 ^a	11.1 \pm 0.8	11 \pm 2.1 ^b	12.6 \pm 3.3
After 4 weeks	8.6 \pm 0.5	11.8 \pm 3.1	13.7 \pm 2.7	7.8 \pm 2.1
After 6 weeks	16.9 \pm 1.5	6.2 \pm 0.8 ^a	13.2 \pm 2.4	12.7 \pm 2.3 ^b
After 8 weeks	16.8 \pm 1.4 ^a	13 \pm 2.8	5.7 \pm 3.4 ^b	18 \pm 3.2
After 10 weeks	11.9 \pm 1.8	13.6 \pm 2.1	14.2 \pm 2.5	15.6 \pm 2.1
After 12 weeks	13.8 \pm 1.6	15 \pm 1.8	12.9 \pm 1.8	14.1 \pm 2

Results expressed as mean \pm SE

Means within rows with different super script indicate significant difference at $p<0.05$

Ingestive and standing idle behaviours were measured as time elapsed for each calf in minutes

The effect of nZnO supplementation on serum zinc is presented in Table (4). It was clear from the presented findings that the level of serum zinc was significantly higher ($p < 0.05$) in the treated group ($9.2 \pm 0.4 \mu\text{g/dl}$) than control group ($8.0 \pm 0.2 \mu\text{g/dl}$) after the 12th week of experiment, while there was no significant difference ($p > 0.05$) at the other periods of the experiment. The obtained results may be related to the greater absorption of Nanozinc from the intestine. It has been shown that Nano particles are absorbed in duodenum by active transport and Nano elemental forms can cross the small intestine and further distribute into the blood (Hillyer and Albrecht 2001). The transition from micro particles to Nano particles ($< 100\text{nm}$ in diameter) involves an increment of surface area so large surface area of the nano particles allows greater solubility which may lead to better utilization in animals (Singh et al., 2018). Additionally, more specific area of high surface zinc oxide nanoparticles leading to the increased mucosal permeability and strong absorption efficiency of small sized zinc (Wijnhoven et al., 2009; Raje et al., 2018). Our findings coincides with that reported by other researchers (Najafzadeh et al., 2013; Rajendran et al., 2013; Li et al., 2016) but are inconsistent with other studies (Zaboli et al., 2013; Wang et al., 2017).

Table (4) Effect of Nano zinc oxide particles on serum zinc level $\mu\text{g/dl}$ in calves.

Experimental period	Control	Treated
Initial	11.4 ± 0.6	11 ± 0.9
After 2 weeks	9.3 ± 1.6	9.5 ± 0.6
After 4 weeks	10.4 ± 0.7	11.5 ± 0.8
After 6 weeks	9.8 ± 1.2	11.5 ± 0.5
After 8 weeks	12.3 ± 0.4	11.6 ± 0.6
After 10 weeks	5.5 ± 0.7	5 ± 0.4
After 12 weeks	8 ± 0.2^b	9.2 ± 0.4^a

Results expressed as mean \pm SE

Means within rows with different super script indicate significant difference at $p < 0.05$

Table (5) Effect of Nano zinc oxide particles on hematological parameters in calves.

Experimental period	Control			Treated		
	Hb (g/dl)	RBCS ($10^{12}/\text{l}$)	PCV (%)	Hb (g/dl)	RBCS ($10^{12}/\text{l}$)	PCV (%)
Initial	12.6 ± 0.8	7.9 ± 0.7	30.6 ± 2.1	13.9 ± 0.5	7.9 ± 1.2	29.8 ± 3.3
After 2 weeks	12.4 ± 0.9	5.4 ± 0.3	20.5 ± 1.1	11.9 ± 0.6	5.3 ± 0.5	20.3 ± 2.3
After 4 weeks	10.9 ± 0.5	4.8 ± 0.06	18.3 ± 0.2	11 ± 0.5	4.9 ± 0.3	18.7 ± 1.3
After 6 weeks	10.2 ± 0.3	5.2 ± 0.4	20.6 ± 0.9	9.8 ± 0.5	4.6 ± 0.3	18.6 ± 1.4
After 8 weeks	9.8 ± 0.5	5.2 ± 0.5	19.9 ± 1.9	10 ± 0.3	4.7 ± 0.2	18.7 ± 1.2
After 10 weeks	10.6 ± 0.6	5.7 ± 0.5	22.4 ± 2.1	10.8 ± 0.3	5.3 ± 0.1	21.3 ± 1
After 12 weeks	10.6 ± 0.4	5.5 ± 0.3	21.5 ± 1.3	10.9 ± 0.4	6.2 ± 0.7	23.5 ± 2.2

Results expressed as mean \pm SE.

Regarding the results in Table (5), it was clear that there was no a significant effect ($p>0.05$) of nZnO particles supplementation on the measured hematological parameters including RBCs count, hemoglobin concentration, and PCV. Similar results were reported in prior researches (Nagalaskmi et al., 2015; Mandal and Dass 2010; Donmez et al., 2002). On the contrary, other investigations (Akbari et al., 2008; Sobhanirad and Naserian 2012) found that nZnO particles supplementation had a pronounced significant effect on some haematological parameters.

CONCLUSION

The effect of nZnO supplementation on growth performance, some behaviours, serum zinc level and some haematological parameters is inconsistent in different studies. In the current study nZnO supplementation may have a little effect on ingestive and standing idle behaviour but it has no pronounced effect on growth performance, serum zinc level and some haematological parameters in calves. Higher doses of nZnO may be required than those used in this experiment in order to induce a significant effect on growth measures, behaviour and serum zinc level. So further studies are required to judge the practical implication and economic feasibility of its use.

REFERENCES

- Ahmadi F., Ebrahimnezhad Y., Sis N. M., and Ghiasi J. (2013). The effects of zinc oxide nanoparticles on performance, digestive organs and serum lipid concentrations in broiler chickens during starter period. *Int J Biosci*, 3(7), 23-29. DOI: [10.12692/ijb/3.7.23-29](https://doi.org/10.12692/ijb/3.7.23-29)
- Akbari, M. R., Kermanshahi, H., Moghaddam, H. N., Moussavi, A. H. and Afshari, J. T. (2008). Effects of wheat-soybean meal based diet supplementation with vitamin A, vitamin E and zinc on blood cells, organ weights and humoral immune response in broiler chickens. *Journal of Animal and Veterinary Advances*, 7(3), 297-304. [Doi=javaa.2008.291.298](https://doi.org/10.21608/javaa.2008.291.298)
- Altman, J. (1974). Observational study of behavior: sampling methods. *Behavior*, (48), 227-267.
- Anil, T.S.V., Venkata Seshaiyah, Ch., Ashalatha, P., and Sudhakar, K. (2019). Effect of Dietary Supplementation of Nano Zinc Oxide (nZnO) on Growth Performance in Crossbred Calves. *International Journal of Current Microbiology and Applied Sciences*, 8(12), 1852-1856. DOI: <https://doi.org/10.20546/ijcmas.2019.812.220>
- Djemali, M. N. (2020). Dairy Livestock Value Chains North Africa Regional Report: Assessment of the National Breeding Objectives and Implementation of Supporting Programs/Initiatives for Dairy Livestock Value Chains. DLVCs, in North Africa.
- Dönmez, N., Hüseyin Dönmez, H., KESKİn, E. R. C. A. N., and ÇELİK, I. (2002). Effects of zinc supplementation to ration on some hematological parameters in broiler chicks. *Biological trace element research*, 87(1), 125-131 DOI: [10.1385/BTER:87:1-3:125](https://doi.org/10.1385/BTER:87:1-3:125)
- Feldman, B.F., Zink, J.G., and Jain, N.C. (2002). A text book of Schalm's veterinary hematology. Philadelphia. Baltimore, New York, London, Buenos Aires, HongKong, Sidney.
- Hayakawa R. Determination of zinc. *Jap J Toxic Environ Health*. (1961). (8), 14-18. Japanese.
- Heisler, G., Fischer, V., de Paris, M., Angelo, I. D. V., Panazzolo, D. M., and Zanela, M. B. (2020). Effect of green tea and oregano extracts fed to preweaned Jersey calves on behavior and health status. *Journal of veterinary behavior*, (37), 36-40. <https://doi.org/10.1016/j.jveb.2020.03.002>

- Hillyer, J. F., and Albrecht, R. M.(2001).Gastrointestinal persorption and tissue distribution of differently sized colloidal gold nanoparticles. *Journal of pharmaceutical sciences*, 90(12), 1927-1936. DOI: [10.1002/jps.1143](https://doi.org/10.1002/jps.1143)
- Jadhav,S.E.(2005). Effect of Different Levels and Sources of Zinc Supplementation on growth, Nutrient utilization, Rumen fermentation, Blood Biochemical and Immune response in Male Buffalo Calves. (Doctoral Dissertation, IVRI).
- Jain, N.C. (1986). Shalm's veterinary hematology. 4th Ed. Lea and Febiger, Philadelphia, U.S.A.
- Kessler, J., Morel, I., Dufey, P. A., Gutzwiller, A., Stern, A., and Geyer, H.(2003). Effect of organic zinc sources on performance, zinc status and carcass, meat and claw quality in fattening bulls. *Livestock Production Science*, 81(2-3),161-171.
[https://doi.org/10.1016/S0301-6226\(02\)00262-2](https://doi.org/10.1016/S0301-6226(02)00262-2)
- Li, M. Z., Huang, J. T., Tsai, Y. H., Mao, S. Y., Fu, C. M., and Lien, T. F.(2016).Nanosize of zinc oxide and the effects on zinc digestibility, growth performances, immune response and serum parameters of weanling piglets. *Animal Science Journal*, 87(11), 1379-1385. DOI: [10.1111/asj.12579](https://doi.org/10.1111/asj.12579)
- Mandal,G.P.(2004). *Effect of Zinc Supplementation on Growth Metabolic Profile and Immunity in Crossbred Calves*. (Doctoral dissertation, IVRI).
- Mandal,G.P., and Dass,R.S.(2010).Haemato-biochemical profile of crossbred calves supplemented with in organic and organic source of zinc .*Indian Journal of Animal Resaersh*,44(3),197-200.
- Marques, R. S., Bohnert ,D. W., Sousa de, O. A., Brandão, A. P., Schumacher, T. F., Schubach, K. M.,Vilela, M. P., Rett, B., & Cooke, R. F. (2019). Impact of 24-h feed, water, or feed and water deprivation on feed intake, metabolic, and inflammatory responses in beef heifers. *Journal of Animal Science* , 97(1), 398-406. doi: 10.1093/jas/sky397.
- Martin, P. and Bateson,p. (1993). *Measuring Behavior. An Introductory Guide*, 2nd ed. Cambridge University Press. Cambridge, UK.
- Mc Dowell,L.R.(1985). *Nutrient requirement of grazing ruminant in warm climate* . Academic press.Inc.California, USA.
- Mishra,A., Swain,R.K., Mishra,S.K., Pnda,N., and Sethy,K.(2014).Growth performance and serum biochemical parameters as affected by Nano zinc supplementation in layer chicks . *Indian J. Anim. Nutr.*,(4),384-388.
- Mousavi-Haghshenas, M. A., Hashemzadeh, F., Ghorbani, G. R., Ghasemi, E., Rafiee, H., and Ghaffari, M. H.(2022).Trace minerals source in calf starters interacts with birth weights to affect growth performance. *ScientificReports*, 12(1),1-13. <https://doi.org/10.1038/s41598-022-23459-4>
- Nagalakshmi, D., and Himabindu, D.(2013).Effect of zinc supplementation from organic and inorganic sources on performance, nutrient utilization and carcass characteristics in lambs. *Indian Journal of Animal Sciences*, 83(4), 411-418.
- Nagalakshmi, D., Sridhar, K., and Parashuramulu, S.(2015).Replacement of inorganic zinc with lower levels of organic zinc (zinc nicotinate) on performance, hematological and serum biochemical constituents, antioxidants status, and immune responses in rats. *Veterinary World*, 8(9), 1156-1162.

DOI: [10.14202/vetworld.2015.1156-1162](https://doi.org/10.14202/vetworld.2015.1156-1162)

Najafzadeh, H., Ghoreishi, S. M., Mohammadian, B., Rahimi, E., Afzalzadeh, M. R., Kazemivarnamkhashti, M., and Ganjealidarani, H., 2013. Serum biochemical and histopathological changes in liver and kidney in lambs after zinc oxide nanoparticles administration. *Veterinary World*, 6(8), 534-537.

DOI: [10.5455/vetworld.2013.534-537](https://doi.org/10.5455/vetworld.2013.534-537)

National Research Council. (2001). Nutrient requirements of dairy cattle: Seventh Revised Edition, 2001. Washington, DC: The National Academies Press.

Radchikov, V. F., Bogdanovich, D. M., Kot, A. N., Tsai, V. P., Tomchuk, V. A., Gryshchenko, V. A., and Trokoz, V. O. (2021). Modern calf rearing system. *Ukrainian Journal of Veterinary and Sciences*, 12(3).

Raje, K., Ojha, S., Mishra, A., Munde, V. K., Rawat, C., and Chaudhary, S. K. (2018). Impact of supplementation of mineral nano particles on growth performance and health status of animals: a review. *J Entomol Zool Stud*, 6, 1690-1694.

Rajendran, D., Kumar, G., Rama krishnan, S., and Thomas, K. S. (2013). Enhancing the milk production and immunity in Holstein Friesian crossbred cow by supplementing novel nano zinc oxide. *Research Journal of Biotechnology*, 8(5), 11-17.

Sahoo, A., Swain, R. K., Mishra, S. K., Behura, N. C., Beura, S. S., Sahoo, C., and Jena, B. (2016). Growth, feed conversion efficiency, and carcass characteristics of broiler chicks fed on inorganic, organic and nano zinc supplemented diets. *Animal Science*, 10(1), 10-18.

SAS. (2008). Statistical analysis system. User Guide, (Release 9.2). SAS Institute Inc. Cary, North Carolina, USA.

Singh, K. K., Maity, S. B., and Maity, A. (2018). Effect of nano zinc oxide on zinc bioavailability and blood biochemical changes in pre-ruminant lambs. *Indian J. Anim. Sci*, 88, 805-807.

Sobhanirad, S., and Naserian, A. A. (2012). Effects of high dietary zinc concentration and zinc sources on hematology and biochemistry of blood serum in Holstein dairy cows. *Animal Feed Science and Technology*, 177(3-4), 242-246.
<https://doi.org/10.1016/j.anifeedsci.2012.06.007>

Swain, P. S., Rao, S. B. N., Rajendran, D., Poornachandra, K. T., Lokesh, E., and Kumar, R. D. (2019). Effect of Nanozinc Supplementation on Haematological and Blood Biochemical Profiles in Goats. *International Journal of Current Microbiology and Applied Sciences*, 8(09), 2688-2696.

DOI: <https://doi.org/10.20546/ijcmas.2019.809.310>

Swain, P. S., Rao, S. B., Rajendran, D., Dominic, G., and Selvaraju, S. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Animal Nutrition*, 2(3), 134-141.

<https://doi.org/10.1016/j.aninu.2016.06.003>

Tietz, N. W. (1976). Fundamentals of clinical chemistry, 2nd ed, W. B. Saunders Co., Philadelphia, p4111.

Wang, C., Zhang, L., Su, W., Ying, Z., He, J., Zhang, L., Zhong, X., Wang, T., and Mishra, Y. K. (2017). Zinc oxide nanoparticles as a substitute for zinc oxide or colistin sulfate: effects on growth, serum enzymes, zinc deposition, intestinal morphology and epithelial barrier in weaned piglets. *PloS one*, 12(7), e0181136. DOI: [10.1371/journal.pone.0181136](https://doi.org/10.1371/journal.pone.0181136)

- Wijnhoven, S. W., Peijnenburg, W. J., Herberts, C. A., Hagens, W. I., Oomen, A. G., Heugens, E. H., and Geertsma, R. E.(2009).Nano-silver–a review of available data and knowledge gaps in human and environmental risk assessment. *Nanotoxicology*, 3(2), 109-138.<https://doi.org/10.1080/17435390902725914>
- Wright, C. L., and Spears, J. W.(2004).Effect of zinc source and dietary level on zinc metabolism in Holstein calves. *Journal of Dairy Science*, 87(4), 1085-1091. DOI: [10.3168/jds.s0022-0302\(04\)73254-3](https://doi.org/10.3168/jds.s0022-0302(04)73254-3)
- Zaboli, K., Aliarabi, H., Bahari, A. A., and Abbas, A. K. R. (2013).Role of dietary nano-zinc oxide on growth performance and blood levels of mineral: A study on in Iranian Angora (Markhoz) goat kids.*Journal of Pharmaceutical Health Sciences*, 2(1), 19-26.
- Zhao, C. Y., Tan, S. X., Xiao, X. Y., Qiu, X. S., Pan, J. Q., and Tang, Z. X.(2014).Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. *Biological trace element research*, 160(3), 361-367. DOI: [10.1007/s12011-014-0052-2](https://doi.org/10.1007/s12011-014-0052-2)