Effect of Different Levels and Sources of Supplemental Nano Zinc on Blood-Biochemical Profile and Serum Mineral Status in Wistar Rats (*Rattus norvegicus*)

Kanti Raje[1], Anil K. Garg[1], Sunil E. Jadhav[1], Narayan Dutta[1], Brijesh K. Ojha[1,2] and Alok Mishra[1]

[1]*Centre of Advanced Faculty Training in Animal Nutrition, ICAR-Indian Veterinary Research Institute, Izatnagar, INDIA*
[2]*Department of Animal Nutrition, College of Veterinary Science & Animal Husbandry, Rewa, NDVSU, Jabalpur (MP), INDIA*

*Corresponding author: BK Ojha ; Email: drbrijesh.ojha@gmail.com

ABSTRACT

This study examined the suitability and comparative efficacy of Zn through different levels and sources on blood-biochemical profile and serum mineral status. 63 weaned (130±3.1g) wistar rats (*Rattus norvegicus*) divided into 7 equal groups in completely randomized design. These rats were fed a common basal (synthetic) diet for 90 days, except for Zn supplementation, which was 10 ppm through zinc sulphate or 10, 20 and 40 ppm nano Zn either from commercial or synthesized (prepared by green method) source. At the end of the experiment, blood samples were collected from each animal through cardiac puncture and analysed for hemato-biochemistry and serum minerals. The Hb, PCV in blood and glucose, urea, total protein, albumin, globulin and creatinine in serum were comparable (P>0.05) among various treatment groups. Serum Ca, P, Cu, Fe and Mn were also not affected by source and different levels of Zn supplementation, however, serum Zn levels were significantly (P<0.001) higher in nano zinc supplemented groups as compared to control and 10 ppm nano Zn groups. It is concluded that Zn nano particles can be safely supplemented up to 40 ppm level in the diet of rats.

Keywords: Nanoparticle, hemato-biochemical, synthetic diet, wistar rat

Zinc (Zn) is the second most abundant trace element in the animal body. It can't be stored in the body (Zalewski *et al.*, 2005) and requires regular dietary intake to meet the physiological needs. Zinc (Zn) is an essential micronutrient for every living being and involved in a variety of biological functions (Underwood and Suttle, 1999). It is involved in several metabolic pathways as cofactors in many enzyme systems (more than 300) and component of a large number of metallo-enzymes; as a proton donor at the active site of an enzyme, and as a bridging atom between the substrate and the enzyme (Kaneko *et al.*, 2008) like carbonic anhydrase, which is required to transfer carbon dioxide in the red blood cells. Early work suggested that Zn deficiencies can affect growth, reproduction, immune system and gene expression in ruminants (Underwood and Suttle, 1999). The need for Zn by most animals is based on its influence on enzymes and proteins and their activities. These enzymes and proteins affect vitamin A synthesis, collagen fiber degradation, free radical destruction,

membrane stability of red blood cells, metabolism of essential fatty acids, carbohydrate metabolism, protein synthesis, and metabolism of nucleic acids, among others (Powell, 2000; McCall *et al.,* 2000; Stefanidou *et al*., 2006; Rubio *et al.,* 2007).

The major source of zinc for animal feed supplementation has been its inorganic salts, such as Zn-sulphate, Zn-oxide and Zn-chloride. However, their bioavailability has been reported to be quite low, hence required in higher amount (Suttle, 2010; Hill *et al.*, 2014; Zhao *et al*., 2014; Huang *et al*., 2016). Recent studies showed that nanoparticles of mineral elements have higher bioavailability, because of their novel characteristics (Swain *et al.*, 2016; Tsai *et al.*, 2016), such as, greater specific surface area, higher surface activity, high catalytic efficiency and stronger adsorbing ability (Rajendran *et al.*, 2013; Sheikh *et al*., 2016). These are stable even under high temperature and pressure and can be easily taken up by the gastrointestinal tract and

utilized in the animal system, making them more effective than the larger sized particles. The intrinsic properties of nano metals are mainly determined by its size, shape, composition, crystalline structure, and morphology. The functional activities such as chemical, catalytic or biological effects of nano minerals are heavily influenced by their particle size. It has been reported that nanoparticle showed new characteristics of transport and uptake and exhibit higher absorption efficiencies (Zha *et al*., 2008; Liao *et al*., 2010; Chaudhry and Castle, 2011; Albanese *et al*., 2012; Rajendran *et al*., 2013) and reaches deeper into tissues and may enhance gene expression (Zhang *et al*., 2016). The rapid biological synthesis of zinc nanoparticles using green synthesis method provides an environmental friendly, simple, economic, non-toxic and efficient route for synthesis of nanoparticles. Until date, not much information is available on the suitability and efficacy of nanoparticle forms of minerals including that of Zn in the diet of the animals (Ahmadi *et al.*, 2013). Therefore, the present study was conducted to test the suitability and efficacy of Zn nanoparticle at different levels on blood-biochemical profile and serum mineral status.

MATERIALS AND METHODS

Animals, housing and management

Sixty three weaned healthy male wistar rats (*Rattus norvegicus*) were procured from Laboratory Animal Research Section of I.V.R.I, Izatnagar. This experiment was approved by the "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA), India. Rats were adapted to the new environment for a period of 7 days by keeping them on a standard diet before the start of the actual experiment. These animals were then divided into seven groups of 9 animals in each group having three replicates of three animals in each replicate on the basis of their body weight (130.2±3.1g) following completely randomized design. All the experimental animals were housed in a well-ventilated room adopting strict management and hygienic practices throughout the experiment.

Feeds and feeding

All the experimental animals were offered a common synthetic (Zn free) diet as per NRC (1995) with the supplementation of different levels of Zn from different sources. The rats in control group (CON) were offered 10 ppm Zn diet through $ZnSO_4$; however, rats in group C10, C20 and C40 were fed diets having 10, 20 and 40 ppm, respectively through commercial nano Zn (SRL Pvt. Ltd.). Rats in group GS10, GS20 and GS40 were provided diets having 10, 20 and 40 ppm, respectively through nano Zn synthesized by using green synthesis method.

Table 1: Physical composition of synthetic diet

Attributes	Composition (percent)
Corn Starch	40
Casein	25
Sucrose	20
Cellulose	5
Oil	5
Mineral Mixture (Without Zinc)	3.5
Vitamin Mixture	1
Choline Chloride	0.3
DL- methionine	0.2

Throughout the experimental period (90 days), clean drinking (RO) water was provided *ad libitum* twice a day daily and weighed amount of diet was offered daily at 9.30 AM and residue left was collected after 24 hrs. The amount of feed offered was regularly revised at weekly interval as per body weight of animals. After the completion of experiment, blood samples were collected from each animal and kept into two part, 1st with anticoagulant for haematological value and 2nd one for separation of serum and analysed serum biochemical parameter and different mineral status.

Chemical analysis

Samples of synthetic diet was milled to pass through a 1.0 mm sieve and then analysis was done as per Association of Official Analytical Chemist (AOAC, 2012) methods to determine dry matter (DM) by the oven drying method, organic matter (OM) by muffle furnace incineration, crude protein by Kjeldahl method (N×6.25), ether extract, ash. The calcium was estimated following the method of Talapatra *et al*. (1940) and phosphorus was estimated as per AOAC (2012) using UV- visible spectrophotometer. Trace minerals in feed was estimated by atomic absorbance spectrophotometer [Model 4141, Electronic Corporation

Blood analysis

Collection of blood and separation of serum

After the completion of experiment, blood samples were collected from each animal through cardiac puncture in morning (before watering and feeding) at the time of slaughter, out of which 5 ml blood was taken into clean and dry test tube and kept in slanting position for 45 minutes to separate the serum. Another 2 ml blood sample was taken in clean and dry micro centrifuge tube (1-2 ml) containing anticoagulant (EDTA). The blood samples were brought to the laboratory and centrifuged at 3000 rpm for 15 min to separate serum and collected in small plastic eppendorf tubes (2ml) and stored at -20^0 C for further analysis

Estimation of hematological and serum biochemical constituents

Hemoglobin (g/dl), packed cell volume (PCV %), red blood cell (RBC, 10^9/L), white blood cells (WBC, 10^9/L), lymphocyte (LY, 10^9/L), monocyte (MO,10^9/L) and granulocyte (GR, 10^9/L) in the blood samples were analysed using HA-22/20/ Vet Hematology Analyzer (Clindiag system B.V.B.A) as per manufacturer protocol.

The serum samples were analyzed for different biochemical constituents viz. glucose, total protein, albumin, globulin, urea, creatinine and cholesterol using diagnostic kits manufactured by CREST BIOSYSTEMS, A Division of Coral Clinical Systems, Goa, India.

Estimation of minerals in serum samples

Calcium and phosphorus in serum was estimated by using kit manufactured by Span Diagnostics Limited, Surat, Indiaand concentration of trace minerals viz. zinc, copper, iron, manganese in serum and tissue samples were estimated by atomic absorbance spectrophotometer (AAS), Model no. 4141, Electronic Corporation of India (Hyderabad, India).

Statistical Analysis

The experimental data generated on blood hemato-

biochemical and serum mineral profile were analyzed using one way ANOVA (statistical package SPSS 20.0) and means were compared by Duncan's multiple range test was used to compare difference among the treatments the P value less than 0.05 was taken to indicate statistical significance by adopting standard statistical procedures (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Chemical composition of the feeds

The chemical composition of the basal (synthetic) diet used for feeding of rats is presented in Table 2. The proximate composition of the basal diet offered to the wistar rats in the present experiment including the level of CP (19.54%) in the basal diet showed that it was almost as per NRC (1995) standard.

Table 2: Chemical composition of the experimental basal (synthetic) diet

Nutrients Composition	(% DM basis)
Organic matter	96.38
Crude protein	19.54
Ether extract	4.91
Total Carbohydrates	71.93
Total ash	3.62
Acid insoluble ash	0.22
Calcium	0.48
Phosphorus	0.29
Copper (ppm)	5.72
Iron (ppm)	45.00
Manganese (ppm)	9.32

Hematological and Serum biochemical parameters

The mean values of blood RBC, haemoglobin (Hb), PCV, WBC, lymphocyte, monocyte and granulocyte in different groups of rat are presented in Table 3. Statistically there was no significant (P>0.05) difference in the mean Hb and PCV values among the 7 groups. The values of RBC, WBC, lymphocytes, monocyte and granulocyte counts were also found comparable (P>0.05) among the different groups. The mean values of serum glucose, urea, creatinine, and total cholesterol did not differ (P>0.05)

among the different groups. The mean total protein (TP), albumin and globulin values were found to be comparable (P>0.05) among the different groups. The mean value of albumin: globulin ratio also did not differ (P>0.05) among different groups.

Comparable (P>0.05) blood Hb, PCV, RBC, WBC, lymphocyte, monocyte, granulocyte among different groups (Table 3) indicated that supplementation of nano zinc particles either through commercial or green synthesis at 10, 20 and 40 ppm level in the diet had no effect on these blood parameters in the wistar rats. Serum glucose, urea, creatinine, total cholesterol, total protein, albumin, globulin, A: G ratio were similar among different groups indicating that supplementation of 10, 20, 40 ppm Zn either through inorganic form (ZnSO$_4$) or as its nanoparticles (commercial and green synthesized) in the diet had no effect on these parameters in wistar rats.

Contrary to our observations, Ahmadi *et al.* (2013) observed that supplementation of 30-120 ppm of zinc oxide nano particles to broiler significantly decreased LDL, cholesterol and triglyceride level and significantly increased HDL level as compared to control. Najafzadeh *et al.* (2013) fed 20 mg zinc nano particles per kg body weight daily for 25 days in lambs and found that, except for creatinine, there was no significant change in any of the blood parameters. A higher total protein and lower glucose concentration were observed in broilers supplemented with 80 ppm of organic Zn; however, there was no difference in total cholesterol level compared to control values (Yalcinkaya *et al.*, 2012). This may be due to the high level of Zn supplemented in their study. Uniyal *et al.* (2017) reported no significant difference in blood biochemical parameters of guinea pig fed 20 ppm level nano zinc from different sources. Similarly Shinde *et al.* (2006) found no difference in blood biochemistry of guinea pig supplementing 20 ppm organic Zn or ZnSO$_4$ and broilers supplemented with 25–120 ppm Zn as organic Zn or ZnSO$_4$ (Salim *et al.*, 2012). Gonzales-Eguia *et al.* (2009) reported that supplementation of nano Cu in

Table 3: Hematological parameters of rats in different groups

Attributes	CON	C10	C20	C40	GS10	GS20	GS40	SEM	P value
RBC (109/L)	5.38	4.68	4.97	5.05	4.81	4.83	5.11	0.09	0.431
Hb (g/dL)	12.06	11.57	12.26	12.17	11.73	11.68	12.64	0.16	0.565
PCV (%)	30.93	26.30	28.03	28.74	27.84	26.91	29.72	0.46	0.118
WBC (109/L)	17.74	18.12	19.97	21.02	18.78	18.99	20.35	0.85	0.947
Lymphocyte (109/L)	5.29	4.50	5.62	6.38	5.76	5.85	5.64	0.32	0.838
Monocyte (109/L)	2.45	2.23	2.65	4.25	2.38	2.62	2.46	0.27	0.430
Granulocyte (109/L)	10.07	11.38	11.61	12.68	14.51	11.13	11.95	0.62	0.629
Lymphocyte (%)	25.87	30.02	31.37	32.13	25.07	42.22	31.43	2.46	0.686
Monocyte (%)	12.24	12.33	13.04	13.09	12.37	13.15	12.16	0.44	0.992
Granulocyte (%)	61.90	57.63	55.69	55.14	59.15	55.97	54.10	2.42	0.986

Table 4: Serum biochemical parameters of rats in different groups

Attributes	CON	C10	C20	C40	GS10	GS20	GS40	SEM	P value
Glucose (mg/dL)	102	101	102	105	104	105	104	1.95	0.996
Urea (mg/dL)	21.7	21.3	20.5	21.7	22.7	20.2	18.9	0.62	0.776
Creatinine (mg/dL)	1.73	1.67	1.69	1.67	1.56	1.58	1.63	0.02	0.561
Total cholesterol (mg/dL)	83.0	90.4	80.7	86.1	85.1	88.6	78.8	1.45	0.318
Total protein (g/dL)	7.29	6.86	6.89	6.93	6.79	7.31	6.62	0.09	0.294
Albumin (g/dL)	3.83	3.66	3.59	3.63	3.13	3.50	3.38	0.05	0.052
Globulin (g/dL)	3.46	3.20	3.30	3.30	3.66	3.81	3.24	0.10	0.604
A:G ratio	1.28	1.19	1.14	1.11	0.89	0.99	1.16	0.05	0.55

Table 5: Serum minerals profile of rats in different groups

Attributes	CON	C10	C20	C40	GS10	GS20	GS40	SEM	P value
Ca (mg/dL)	11.0	11.3	11.3	11.2	10.5	11.8	11.5	0.20	0.776
P (mg/dL)	8.59	8.02	8.31	7.93	7.70	7.87	7.39	0.17	0.630
Zn (ppm)*	1.27[a]	1.79[b]	2.07[c]	2.46[cd]	1.66[b]	2.85[d]	2.90[d]	0.11	0.001
Cu (ppm)	1.27	1.07	1.14	1.03	0.95	0.87	1.08	0.06	0.669
Mn (ppm)	0.72	0.64	0.57	0.59	0.47	0.53	0.51	0.03	0.728
Fe (ppm)	7.52	7.07	8.03	9.46	8.01	7.42	8.41	0.37	0.119

[abcd] Means bearing different superscript in a row differ significantly (P<0.001)

piglets at 50 ppm level had no effect on serum cholesterol concentrations, as well as haematology traits like RBC, WBC, MCV, HGB, HCT, PLT and RDW. Kessler *et al*. (2003) too did not find any effect in level of blood Hb, PCV, total protein, albumin and urea in bulls fed different sources of Zn. Thus, it appears that supplementation of Zn from different sources including that of nanoparticles had no effect on serum biochemical and haematological parameters in rats.

Serum mineral profile

Serum Ca, P, Cu, Fe and Mn levels were Comparable (P>0.05) in the different experimental groups (Table 5) indicated that supplementation of nano Zn (either through commercial or synthesized) up to 40 ppm level in the diet had no effect on these serum minerals status. Uniyal *et al.* (2017) also reported no effect on serum minerals (Ca, P, Cu, Fe, and Mn) profile in guinea pigs supplemented with 20 ppm from different sources including nano Zn. Zaboli *et al*. (2013) also reported that serum minerals levels were not affected in kids supplemented 20-40 ppm nano zinc oxide. Similar results were observed in guinea pigs (Shinde *et al*., 2006), lambs (Garg *et al*., 2008), broilers (Feng *et al*., 2009) and goats (Jia *et al*., 2009) supplemented with 20-120 ppm Zn either as organic Zn or through $ZnSO_4$. Similarly, Salim *et al*. (2012) did not observe any difference in serum Ca level in broilers supplemented with 25 ppm organic.

It was observed that the Zn concentration was significantly (P<0.001) increased in all the nano zinc supplemented groups. It was further observed that serum zinc levels were further increased with the increasing level of zinc supplementation, with highest values in group 40 ppm

nano Zn group (Table 5) indicating a positive correlation of dietary Zn nano particles with serum Zn level. Contrary to our observations Carlson *et al*. (2004) reported that there was no effect on serum Zn level in pigs supplemented 50-2000 ppm Zn either from organic or inorganic sources, which may be due to very high level of Zn (165 ppm) in the basal diet itself. However, similar to our observations, Uniyal *et al.* (2017) found that serum Zn concentration was significantly higher in nano group than other Zn sources. Li *et al.* (2016) observed higher serum Zn concentration in nano-Zn and organic-Zn groups than in the ZnO and control groups of weanling piglets supplemented with 120 mg/kg Zn in the diet.

Therefore, it is concluded that nano Zn particles can be safely included in the diet of rats up to 40 ppm level as there was no adverse effect on the blood-biochemical profile and significantly improved the serum Zn levels in rats.

REFERENCES

Ahmadi, F., Ebrrahimnezhad, Y., Sis, M.N. and Ghalehkandi, J.G. 2013. The effects of zinc oxide nanoparticles on performance, digestive organs and serum lipid concentrations in broiler chickens during starter period. *Int. J. Biol. Sci.,* **3**: 23–29.

Albanese, A., Tang, P.S. and Chan, W.C. 2012. The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annu. Rev. Biomed. Eng.,* **14**: 1–6.

AOAC, 2012. Official Method of Analysis, 19th ed. AOAC International, Virginia, USA.

Carlson, M.S., Boren, C.A., Wu, C., Huntington, C.E., Bollinger, D.W. and Veum, T.L. 2004. Evaluation of various inclusion rates of organic zinc either as polysaccharide or proteinate complex on the growth performance, plasma, and excretion of nursery pigs. *J. Anim. Sci.,* **82**: 1359-1366.

Chaudhry, Q. and Castle, L. 2011. Food applications of nanotechnologies: an overview of opportunities and challenges for developing countries. *Trends Food Sci. Technol.,* **22:** 595–603.

Feng, J., Ma, W.Q., Niu, H.H., Wu, X.M., Wang, Y. and Feng. J. 2009. Effects of zincglycine chelate on growth, hematological, and immunological characteristics inbroilers. *Biol. Trace Elem. Res.,* **133(2)**: 203-221.

Garg, A.K., Mudgal, V. and Dass, R.S. 2008. Effect of organic zinc supplementation ongrowth, nutrient utilization and mineral profile in lambs. *Anim. Feed Sci. Technol.,* **144**: 82–96.

Gonzales-Eguia, A., Fu, C.M., Lu, F.Y. and Lien, T.F. 2009. Effects of nano copper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. *Livest. Sci.,* **126**: 122–129.

Hill, G.M., Mahan, D.C. and Jolliff, J.S. 2014. Comparison of organic and inorganic zinc sources to maximize growth and meet the zinc needs of the nursery pig. *J. Anim. Sci.*, **92:** 1582–1594.

Huang, D., Zhuo, Z., Fang, S., Yue, M. and Feng, J. 2016. Different zinc sources have diverse impacts on gene expression of zinc absorption related transporters in intestinal porcine epithelial cells. *Biol. Trace Elem. Res.,* **173**: 325–332.

Jia, W., Zhu, X., Zhang, W., Cheng, J., Guo, C. and Jia, Z. 2009. Effects of source of supplemental zinc on performance, nutrient digestibility and plasma mineral profile in cashmere goats. *Asian Aust. J. Anim. Sci.*, **22(12)**: 1648-1653.

Kaneko, J. J., Harvey, J.W. and Bruss, M.L. 2008. Clinical biochemistry of domestic animals. 6th ed., Academic Press.

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. *Livest. Prod. Sci.,* **81(2)**: 161-171

Li, M.Z., Huang, J.T., Tsai, Y.H., Mao, S.Y., Fu, C.M. and Lien, T.F. 2016. Nano size of zinc oxide and the effects on zinc digestibility, growth performances, immune response and serum parameters of weanling piglets. *Anim. Sci., J.,* **87**: 1379–1385.

Liao, C.D., Hung, W.L., Jan, K.C., Yeh, A.I., Ho, C.T. and Hwang, L.S. 2010. Nano/ sub microsized lignan glycosides from sesame meal exhibit higher transport and absorption efficiency in Caco-2 cell monolayer. *Food Chem.*, **119**: 896–902.

McCall, K.A., Huang, C. and Fierke, C.A. 2000. Function and mechanism of zinc metalloenzymes. *J. Nutr.,* **130**: 1437S-1446S.

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

NRC, 1995. *Nutrient Requirement of Laboratory Animals*. National Academic Press, Washington, DC, USA.

Powell, S.R. 2000. The antioxidant properties of zinc. *J. Nutr.,* **130**: 1447S-1454S.

Rajendran, D., Thulasi, A., Jash, S., Selvaraju, S. and Rao, S.B. 2013. Synthesis and application of nano minerals in livestock industry. In: Animal Nutrition and Reproductive Physiology (Recent Concepts). Satish Serial Publishing House, Delhi, pp. 517–530.

Rubio, C., González, D., Martín-Izquierdo, R.E., Revert, C., Rodríguez, I. and Hardisson, A. 2007. El zinc: oligoelementoessential. *Nutr. Hosp.,* **22**: 101-107.

Salim, H.M., Lee, H.R., Jo, C., Lee, S.K. and Lee, B.D. 2012. Effect of sex and dietary organic zinc on growth performance, carcass traits, tissue mineral content, and blood parameters of broiler chickens. *Biol. Trace Elem. Res*., **147**: 120–129.

Sheikh, A.A., Aggarwal, A. and Aarif, O. 2016. Effect of in vitro zinc supplementation on HSPs expression and Interleukin 10 production in heat treated peripheral blood mononuclear cells of transition Sahiwal and Karan Fries cows. *J. Therm. Biol.,* **56:** 68–76.

Shinde, P.L., Dass, R.S., Garg, A.K., Chaturvedi, V.K. and Kumar, R. 2006. Effect of zinc supplementation from different sources on growth, nutrient digestibility, blood metabolic profile, and immune response of male guinea pigs. *Biol. Trace Elem. Res.*, **112:** 247–262.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods, 8th ed. Iowa State University Press, Iowa. SPSS, 2011.

Stefanidou, M., Maravelias, C., Dona, A. and Spiliopoulou, C. 2006. Zinc: a multipurpose trace element. *Arch. Toxicol*., **80**: 1- 9.

Suttle, N.F. 2010. Mineral Nutrition of Livestock, 4th ed. CABI Publishing, USA.

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. *Anim. Nutr.,* **2**:134–141.

Talapatra, S.K., Ray, S.C. and Sen, K.C. 1940. Estimation of phosphorus, chlorine, calcium, magnesium, sodium and

potassium in foodstuffs. *Indian J. Vet. Sci. Anim. Husb.,* **10**: 243–246.

Tsai, Y.H., Mao, S.Y., Li, M.Z., Huang, J.T. and Lien, T.F. 2016. Effects of nano size zinc oxide on zinc retention, eggshell quality, immune response and serum parameters of aged laying hens. *Anim. Feed Sci. Technol*., **213**: 99–107.

Underwood, E.J. and Suttle, N. F. 1999. The Mineral Nutrition of Livestock. 3rd ed., CABI Publishing, New York, pp-614.

Uniyal, S., Garg, A.K., Jadhav, S.E., Chaturvedi, V.K. and Mohanta, R.K. 2017. Comparative efficacy of zinc supplementation from different sources on nutrient digestibility, hemato-biochemistry and anti-oxidant activity in guinea pigs. *Livest. Sci.,* **204:** 59-64.

Yalcinkaya, İ., Çınar, M., Yıldırım, E., Erat, S., Başalan, M. and Güngör, T. 2012. The effect of prebiotic and organic zinc alone and in combination in broiler diets on the performance and some blood parameters. *Ital. J. Anim. Sci.,* **11(3)**: e55.

Zaboli, K., Aliarabi, H., Bahari, A.A. and Abbasalipourkabir, R. 2013. Role of dietary nano ZnO on growth performance and blood levels of mineral: A study on in Iranian Angora (Markhoz) goat kids. *J. Pharma. Health Sci*., **2(1)**: 19-26.

Zalewski, P.D., Ai, Q.T., Dion, G., Lata, J., Chiara, M. and Richard, E.R. 2005. Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets: a review. *Pharmacol Ther.,* **105**: 127- 49.

Zha, L.Y., Xu, Z.R., Wang, M.Q. and Gu, L.Y. 2008. Chromium nanoparticle exhibits higher absorption efficiency than chromium picolinate and chromium chloride inCaco-2 cell monolayers. *J. Anim. Physiol. Anim. Nutr.*, **92**: 131–140.

Zhao, Y.C., Shu, T.X., Xiao, Y.X., Qiu, S.X., Pan, Q.J. and Tang, X.Z. 2014. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broiler. *Biol. Trace Elem. Res.,* **160**: 361–367.