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

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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, 1$^{st}$ with anticoagulant for haematological value and 2$^{nd}$ 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]
of India Limited (ECIL), Hyderabad, India] in the mineral extracts.

**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°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 hematopoietic 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.

| 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 (ZnSO₄) 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 ZnSO₄ and broilers supplemented with 25–120 ppm Zn as organic Zn or ZnSO₄ (Salim et al., 2012). Gonzales-Eguia et al. (2009) reported that supplementation of nano Cu in

| 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   |

| 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    |
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 ZnSO₄. 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.

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