



Nano Zinc Supplementation in Male Wistar Rats

Akash Mishra et al.

## Nano Zinc Supplementation: Its Influence on Growth Performance, Feed Intake and Haematobiochemical Parameters in Male Wistar Rats

Akash Mishra<sup>2</sup>, Chander Datt<sup>1\*</sup>, Kuldeep Dudi<sup>3</sup>, Shambvi<sup>4</sup> and Digvijay Singh<sup>5</sup>

<sup>1\*</sup>Animal Nutrition Division, ICAR- National Dairy Research Institute, Karnal-132001, Haryana, India

<sup>2</sup>Veterinary Assistant Surgeon, Puri, Odisha, India,

<sup>3</sup>District Extension Specialist, Panipat, Haryana,

<sup>4</sup> Environmental Defence Fund, Cornell University, Ithaca, NY, USA.

<sup>5</sup> Department of Animal Nutrition, College of Veterinary and Animal Science, Banda University of Agriculture and Technology, Banda-121001, Uttar Pradesh, India

\* Correspondence: chandatt@gmail.com

### ABSTRACT

A study was conducted to investigate the effect of supplementation of nano zinc (ZnO-NPs) from zinc oxide source on growth performance, feed intake and hemato biochemical parameters in male Wistar rats. A total of 36 male Wistar rat were taken, where 6 animals were sacrificed at zero days for hemato biochemical studies and rest of the rats were divided into five groups of 6 animals in each. The group T1 was fed purified diet without Zn supplementation. In group T2, 12 ppm inorganic Zn was given while the rats in groups T3, T4 and T5 were supplemented with nano Zn @ 3, 6 and 12 ppm, respectively for five weeks. The average daily gain and feed conversion ratio improved in groups supplemented either with 12 ppm inorganic Zn or with 3, 6 and 12 ppm nano Zn. The plasma ALP activity increased ( $P < 0.05$ ) with increase in ZnO-NPs while there was a reverse trend for AST and ALT activities. The concentration of total cholesterol and triglycerides decreased ( $P < 0.5$ ) due to inorganic or ZnO-NPs addition in the diet, however, the effects of 3 ppm ZnO-NPs addition were comparable to those of 12 ppm inorganic Zn or 6 and 12 ppm of nZn. Therefore, supplementation of 3 ppm nano Zn improved growth performance and biochemical parameters in the male Wistar rats and the results were comparable to the groups given either 12 ppm inorganic Zn or 6 and 12 ppm nano Zn.

**KEYWORDS:** Biochemical parameters, Feed intake, Performance, Wistar rats, ZnO-NPs

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### INTRODUCTION

Zinc is one of the most important essential trace elements for animals and its deficiency is a worldwide phenomenon (Chander Datt and Chhabra, 2005). In all species, Zn deprivation is characterized by inappetence, retardation of growth, skeletal and reproductive disorders (Suttle, 2010). Deficiency of Zn from the extracellular space resulted in decreased activity of deoxythymidine kinase and reduced levels of adenosine tri phosphate. Hence, Zn may directly regulate DNA synthesis (Mc Donald, 2000). The acute Zn deprived rats were unable to nibble while little addition of Zn (3-6 ppm) avoided this complication (Apgar et al., 1993). Zinc deficient rats (1 ppm) showed decrease ( $P < 0.05$ ) in body weight gain and feed intake and showed reduced growth with bone abnormalities (Roth, 2003 and Cho et al., 2013). The optimum growth and feed intake was

observed in rats with 12 ppm Zn supplementation as  $ZnCO_3$  (Nagalakshmi et al., 2013) and no further beneficial effects were observed on performance with increase in Zn supplementation (24 to 48 ppm). The organic Zn supplemented in nicotinate form (6, 9 and 12 ppm) in female Sprague Dawley rats had no significant effect on body weight gains and daily feed intake (Nagalakshmi et al., 2015). Zinc-deficient diet groups (19 mg/kg diet, 1/2 of control and 3.8 mg/kg diet, 1/10 of control) gained less ( $P < 0.05$ ) than the control groups (38 mg/kg diet, control) in growing male and female rats for 10 weeks (El Hendy et al., 2010).

In India, common practice is to supplement inorganic mineral sources like sulphates, carbonates and chloride forms (Batal et al., 2001). Interaction of inorganic forms with other nutrients and minerals often leads to higher level of supplementation for

better performance which might result in toxicity and environmental pollution (Cheng et al., 1998). Recently, organically complexed or chelated forms of minerals are being used for livestock because of higher bioavailability (Edwards and Baker, 1999). However, with the advent of nanotechnology, it is possible to use nanoparticle as a source of mineral supplement in animal rations. The nano sized minerals increased absorption (Davda and Labhasetwar, 2002). Numerous interfaces where the atomic configurations differ from those of the crystal lattice define nanoparticles. (Ghosh, 2012). There is scanty information on ZnO-NPs supplementation in animals. Hence, this work was undertaken to examine the effect of ZnO-NPs addition in the diet on growth performance and biochemical parameters in male Wistar rats.

## MATERIALS AND METHODS

### Animal selection

Thirty-six weaned healthy Wistar male rats (*Rattus norvegicus*) weighing  $111 \pm 0.5$ g were selected from Small Animal House, ICAR-National Dairy Research Institute, Karnal, Haryana, India and adapted to the new environs by keeping them for 7 day on a normal diet prior to the start of the actual trial. At zero day, six animals were sacrificed for collection of blood for estimation of hematobiochemical parameters. Rats were divided into five groups of six animals each i.e. T1 (fed with purified diet without Zn supplementation); T2 (fed as per group T1 supplemented with inorganic Zn at 12 ppm level); T3 (fed as per group T1 supplemented with nano Zn at 3 ppm level); T4 (fed as per group T1 supplemented with nano Zn at 6 ppm level) and T5 (fed as per group T1 supplemented with nano Zn at 12 ppm level). The Institutional Animal Ethics Committee (IAEC) of ICAR-NDRI, Karnal, Haryana approved the study and the protocol was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Environment, Forests and Climate Change, Government of India.

### Housing and feeding management

All the animals were caged in group of two animals and housed in a well-ventilated room (22-

25°C, 40-60% relative humidity, 12-hour light/dark cycle) adopting strict management and hygienic practices throughout the experiment. Clean drinking water was provided *ad lib.* throughout the experiment in nipple fitted polypropylene bottles. The animals were offered a common Zn free purified diet according to ICAR (2013) for a period of 35 days (Table 1). The experimental feeding was similar in all five groups except for the sources of Zn. The feed intake and body weights of rats were recorded weekly. Analysis of basal diet was done for proximate principles and minerals (AOAC, 2005).

Table 1. Composition of purified diet

| Ingredient         | Proportion |
|--------------------|------------|
| Sucrose            | 45.0       |
| Casein             | 20.0       |
| Corn starch        | 15.0       |
| Corn oil           | 5.0        |
| Cellulose          | 5.0        |
| Mineral premix*    | 3.5        |
| Vitamin premix**   | 1.0        |
| DL-Methionine      | 0.3        |
| Choline bitartrate | 0.2        |
| Wheat flour        | 5.0        |

\*Mineral premix(g/kg): Calcium phosphate, dibasic (CaHPO<sub>4</sub>) (500.00); Potassium citrate, monohydrate (K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·H<sub>2</sub>O) (220.00); Sodium chloride (NaCl) (74.00); Potassium sulphate (K<sub>2</sub>SO<sub>4</sub>) (52.00); Magnesium oxide (MgO) (24.00); Ferric citrate (6.00); Manganous carbonate (MnSO<sub>4</sub>) (3.50); Zinc carbonate (ZnCO<sub>3</sub>)<sup>§</sup> (1.60); Chromium potassium sulphate (CrK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O) (0.55); Cupric carbonate (CuCO<sub>3</sub>) (0.30); Potassium iodate (KIO<sub>3</sub>) (0.01); Sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O) (0.01); Sucrose powder(118.03).

§Only added in mineral mixture supplemented to animals of group T<sub>2</sub>.

\*\*Vitamin premix (g/kg): Nicotinic acid (3.000); Calcium d-pantothenate (1.600); Pyridoxine hydrochloride (0.700); Thiamin hydrochloride (0.600); Riboflavin (0.600); Folic acid (0.200); d-Biotin (0.020); Cyanocobalamine (0.001); Retinyl acetate (400,000 IU); Alfa-tocopheryl acetate (5000 IU); Cholecalciferol (0.0025); Menaquinone (0.005); Sucrose powder (To make <1000 g).

### Haematological and biochemical parameters

At the beginning and end of the experiment, blood samples were collected from the sacrificed animals after being slightly anesthetized with diethyl ether. One mL of blood was anti coagulated with EDTA shortly after collection for analysis of red blood cell (RBC), haemoglobin (Hb), haematocrits (PCV), total leukocyte count (TLC), mean corpuscular hemoglobin (MCH %), mean corpuscular volume (MCV %) and mean corpuscular hemoglobin concentration (MCHC %) by an automatic hematology cell counter (Nihon Kohden, Celltaca, Tokyo, Japan). Four mL of blood was transferred into sterile collection tubes without any addition of anticoagulant. The samples were centrifuged (Sigma, Laborzentrifugen, 3K15) at 5000 rpm for 10 min. at 4°C. The serum samples were collected, stored at -20°C and used for analysis of different biochemical constituents (glucose, total protein and albumin, globulin, cholesterol, triglyceride, ALP, AST, ALT) using diagnostic kits (Recombigen Laboratories Pvt. Ltd, India).

### Statistical analysis

Statistical analysis of data was carried out by one way analysis of variance (ANOVA) model (Snedecor and Cochran, 1994). This statistical ANOVA model was incorporated with General Linear Models (GLM) procedure of SPSS 16.0 computer packages. Comparison of data was done with Tuckey's honest significant difference (HSD) test method (Steel and Torrie 1980).

## RESULTS AND DISCUSSION

### Body weights, average daily gain and feed conversion ratio in different groups

The DM, OM, CP, CF, EE, total ash and NFE contents of purified diet were found to be 95.08±1.06, 96.93±0.01, 19.50±0.08, 5.22±0.05, 4.89±0.08, 3.06±0.01 and 67.85±0.08 %, respectively. The Ca and P level was found to be 0.70±0.05 and 0.37±0.02 % in purified diet. The concentration of Zn, Cu, Fe

and Mn was 1.76±0.29, 6.92±0.15, 40.61±0.73 and 10.10±0.87 ppm, respectively. The DM, OM, CP, CF, EE, total ash and NFE content of purified diet were found in prescribed range of nutrient requirement for rats (ICAR, 2013). The average initial body weights of the experimental rats were recorded to be 111.43±0.36 g. At the end of 5<sup>th</sup> week of the experiment, average body weights of rats were similar in all the groups (Table 2). There was no difference in average daily feed intake among the groups (Table 2). The average feed intake varied from 16.77 to 18.45 g/d. The feed conversion ratio improved in groups T2, T3 and T5. The significantly highest FCR value was seen in group T1 and lower FCR value in group T5 (Fig. 1). There was no beneficial effect of total replacement of Zn (12 ppm) from ZnCO<sub>3</sub> with other organic sources (Zn-Met, Zn proteinate and Zn propionate) on feed intake in rats (Nagalakshmi et al., 2012). Further, it was reported that organic Zn (zinc nicotinate) supplementation (6, 9 and 12 ppm) compared to inorganic Zn (12 ppm) in female Sprague Dawley rats had no significant effect on daily feed intake (Nagalakshmi et al., 2012). Zn deficiency in animals might reduce appetite by impairing the taste because the taste is mediated through salivary Zn dependant polypeptide gustin (Droke et al., 1993). Zinc deficient rats (1 ppm) showed significant (P<0.05) decrease in food intake (Cho et al., 2003) compared with Zn adequate rats (35 ppm).

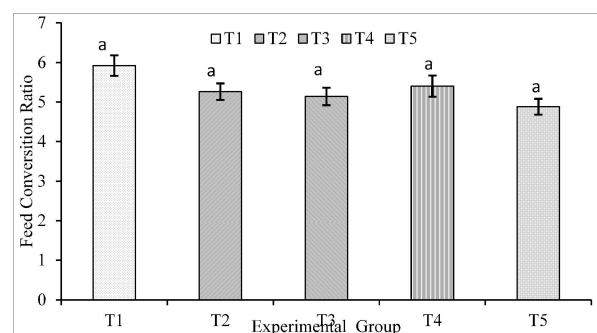


Fig. 1 Feed conversion ratio in rats supplemented with different levels and sources of Zn.

Table 2. Growth performance and feed intake (g) of male Wistar rats supplemented with different levels and sources of Zn

| Week            | Body weight (g)               |                            |                            |                            |                           |
|-----------------|-------------------------------|----------------------------|----------------------------|----------------------------|---------------------------|
|                 | T1                            | T2                         | T3                         | T4                         | T5                        |
| Zero            | 111.00±5.70                   | 110.50±7.02                | 111.67±5.61                | 112.67±5.07                | 111.33±5.46               |
| 1               | 130.50±5.32                   | 131.83±6.38                | 135.00±5.67                | 134.08±5.30                | 134.58±5.09               |
| 2               | 151.33±5.98                   | 155.17±5.24                | 160.75±4.78                | 156.67±4.18                | 161.17±5.64               |
| 3               | 171.68±6.28                   | 179.73±4.66                | 188.52±5.33                | 183.02±4.88                | 188.38±4.80               |
| 4               | 192.60±6.41                   | 204.25±4.43                | 214.50±5.67                | 208.32±5.44                | 214.42±5.10               |
| 5               | 211.90 <sup>b</sup> ±6.63     | 229.33 <sup>ab</sup> ±4.59 | 239.36 <sup>a</sup> ±5.99  | 234.01 <sup>ab</sup> ±5.55 | 239.51 <sup>a</sup> ±5.62 |
| Overall average | 161.50±6.29                   | 168.47±7.20                | 174.46±7.75                | 171.90±7.32                | 174.90±7.75               |
|                 | Average daily feed intake (g) |                            |                            |                            |                           |
| 1               | 18.73 ± 0.47                  | 19.82 ± 0.58               | 20.90±0.55                 | 19.58±0.51                 | 19.42±0.63                |
| 2               | 16.60 <sup>c</sup> ± 0.56     | 17.42 <sup>bc</sup> ± 0.56 | 19.12 <sup>ab</sup> ± 0.69 | 20.51 <sup>a</sup> ±0.57   | 19.58 <sup>ab</sup> ±0.71 |
| 3               | 16.90 <sup>b</sup> ± 0.56     | 15.65 <sup>b</sup> ± 0.58  | 18.86 <sup>a</sup> ±0.45   | 17.21 <sup>ab</sup> ±0.45  | 18.07 <sup>a</sup> ±0.58  |
| 4               | 11.87±0.73                    | 15.81±0.84                 | 12.95±0.89                 | 12.27±1.35                 | 12.27±1.32                |
| 5               | 19.75±0.55                    | 18.75 ± 0.57               | 20.40 ± 1.01               | 21.43±0.69                 | 19.59±0.78                |
| Overall average | 16.77±0.56                    | 17.49±0.40                 | 18.45±0.61                 | 18.20±0.69                 | 17.79±0.63                |
|                 | Average daily gain (g)        |                            |                            |                            |                           |
| 1               | 2.79±0.09                     | 3.05±0.20                  | 3.33±0.17                  | 3.06 ± 0.26                | 3.32±0.14                 |
| 2               | 2.98 <sup>b</sup> ±0.15       | 3.33 <sup>ab</sup> ±0.25   | 3.68 <sup>ab</sup> ±0.18   | 3.23 <sup>ab</sup> ±0.18   | 3.80 <sup>a</sup> ±0.19   |
| 3               | 2.91 <sup>b</sup> ±0.15       | 3.51 <sup>ab</sup> ±0.14   | 3.97 <sup>a</sup> ±0.19    | 3.76 <sup>a</sup> ±0.16    | 3.89 <sup>a</sup> ±0.14   |
| 4               | 2.99 <sup>b</sup> ±0.15       | 3.50 <sup>ab</sup> ±0.14   | 3.71 <sup>a</sup> ±0.11    | 3.61 <sup>ab</sup> ±0.23   | 3.72 <sup>a</sup> ±0.11   |
| 5               | 2.76 <sup>b</sup> ±0.15       | 3.58 <sup>a</sup> ± 0.06   | 3.55 <sup>a</sup> ±0.12    | 3.67 <sup>a</sup> ±0.17    | 3.58 <sup>a</sup> ±0.14   |
| Overall average | 2.88 <sup>b</sup> ±0.06       | 3.40 <sup>a</sup> ±0.08    | 3.65 <sup>a</sup> ± 0.08   | 3.47 <sup>a</sup> ±0.10    | 3.66 <sup>a</sup> ±0.07   |

<sup>a, b, c</sup> Means with different superscripts in a row differ significantly ( $P < 0.05$ )

Rats fed with a Zn-deficient diet consumed less feed and showed reduced growth rate (Yamaguchi and Yamaguchi, 1986; Blanchard et al., 2001). Similar findings were documented (Lina et al., 2009; Ahmadi et al., 2013) who showed that nano Zn supplementation resulted in better growth performance in broilers. No effect was observed in goat kids (Ahmadi et al., 2013) and piglets (Jianbo et al., 2013) due to nano Zn supplementation which could be due to differences in species, supplementary level of Zn and other dietary and managerial conditions. Similar results (Nagalakshmi et al., 2015)

were reported in rats supplemented with 12 ppm Zn as inorganic source ( $ZnCO_3$ ) and no further beneficial effect was observed in weight gain with increased Zn supplementation (24-48 ppm). Replacement of  $ZnCO_3$  (12 ppm) with other organic sources (Zn Met, Zn proteinate and Zn propionate) had no significant effect on body weight of rats (Nagalakshmi et al. 2014; Nagalakshmi et al., 2015). However, Zn supplementation showed higher growth which might be due to the fact that Zn is a part of deoxythymidine kinase which is involved in DNA synthesis and nucleic acid metabolism, therefore,

growth may be hampered in case of Zn deficiency (Suttle, 2010). This was consistent with the results where similar growth rate was found in weanling piglets supplemented with 120 mg/kg of nano-Zn, organic-Zn or ZnO in the diet (Li et al., 2016). There was improvement ( $P < 0.05$ ) in FCR due to supplementation of Zn. A better FCR value due to inorganic Zn supplementation which corroborated our findings (Nagalakshmi et al., 2012). In this study, supplementary nano Zn showed better daily average feed intake at a lesser dose which might be due to better absorption by intestinal villi (Hett, 2004; Cho et al., 2013).

### Effects on haematological parameters

The Hb concentration ranged from  $10.05 \pm 0.35$  to  $11.15 \pm 0.26$  g/dL and RBC values from  $9.30 \pm 0.27$  to  $9.97 \pm 0.25$  million/ $\text{mm}^3$ . The WBC values varied from  $9.41 \pm 0.39$  to  $10.69 \pm 0.31$  thousand/ $\text{mm}^3$  while PCV values ranged from  $35.50 \pm 0.95$  to  $38.50 \pm 1.25\%$ . The values of MCV and MCH varied

from  $37.93 \pm 1.10$  to  $39.83 \pm 1.72\%$  and from  $10.82 \pm 0.53$  to  $11.67 \pm 0.48\%$ , respectively. The MCHC values ranged from  $28.19 \pm 0.87$  to  $30.34 \pm 0.49\%$ . The values of various haematological parameters were similar in all the treatment groups (Table 3). Haematological parameters of Wistar rats were not affected by source and different levels of nZn supplementation (Raje et al., 2018). There was no significant effect of 100% replacement of  $\text{ZnCO}_3$  with various sources of organic Zn (Zn-Met, Zn propionate and Zn propionate) on serum haematological constituents in rats (Nagalakshmi et al., 2014; Nagalakshmi et al., 2016). Organic Zn (Zn nicotinate) supplementation (6, 9 and 12 ppm) in female Sprague Dawley rats did not affect haematological constituents (Nagalakshmi et al., 2015). However, Zn deficiency (19 and 3.8 mg Zn/kg diet) in growing male and female rats for 10 weeks decreased the levels of Hb, total erythrocyte count and PCV while increasing TLC count (El Hendy et al., 2001).

Table 3. Haematological constituents in male Wistar rats supplemented with different levels and sources of Zn

| Parameter                         | Group            |                  |                  |                  |                  |
|-----------------------------------|------------------|------------------|------------------|------------------|------------------|
|                                   | T1               | T2               | T3               | T4               | T5               |
| Haemoglobin (g/dL)                | $10.85 \pm 0.43$ | $11.15 \pm 0.17$ | $10.90 \pm 0.12$ | $10.05 \pm 0.35$ | $11.15 \pm 0.26$ |
| RBC ( $10^6 \times \text{mm}^3$ ) | $9.68 \pm 0.18$  | $9.97 \pm 0.25$  | $9.59 \pm 0.15$  | $9.30 \pm 0.27$  | $9.52 \pm 0.23$  |
| WBC ( $10^3 \times \text{mm}^3$ ) | $9.41 \pm 0.39$  | $10.59 \pm 0.28$ | $10.69 \pm 0.31$ | $10.19 \pm 0.29$ | $10.61 \pm 0.14$ |
| PCV (%)                           | $38.50 \pm 1.25$ | $37.75 \pm 0.62$ | $36.50 \pm 0.50$ | $35.50 \pm 0.95$ | $36.75 \pm 0.75$ |
| MCV (%)                           | $39.83 \pm 1.72$ | $37.93 \pm 1.10$ | $38.04 \pm 0.29$ | $38.27 \pm 1.78$ | $38.67 \pm 1.36$ |
| MCH (%)                           | $11.23 \pm 0.58$ | $11.20 \pm 0.29$ | $11.36 \pm 0.13$ | $10.82 \pm 0.53$ | $11.72 \pm 0.35$ |
| MCHC (%)                          | $28.19 \pm 0.87$ | $29.54 \pm 0.22$ | $29.87 \pm 0.46$ | $28.31 \pm 0.74$ | $30.34 \pm 0.49$ |

### Effect of dietary nano Zn on blood biochemical parameters

The lowest value of serum glucose concentration was found in group T1 and the highest in Zn supplemented groups. The highest value of total protein was seen in groups T5 whereas the lowest level was found in group T1. The values in groups T2, T3, T4 and T5 were comparable. The serum albumin level was the highest in group T5 without any difference with group T2 and T3. The lowest albumin level was observed in group T1. The serum globulin level in different groups ranged from

$3.56 \pm 0.18$  to  $3.78 \pm 0.29$  g/dL, respectively. The A/G ratio was similar in all the groups. The group T1 showed the lowest values of serum ALP and there was no difference among T2, T3, T4, and T5 groups. The highest AST activity was seen in group T1 and the lowest in group T4 and T5. The serum ALT level was highest in group T1 and lowest in groups T2, T3, T4 and T5. The lowest serum cholesterol level was found in group T3, T4 and T5 whereas the highest value was seen in group T1. The values of serum triglyceride levels were lower in groups T2, T3, T4 and T5 compared to group T1.

The increased glucose level in Zn supplemented groups in this study was supported (Mishra et al., 2014). There was decrease in glucose, total protein and albumin level in group T1 (El Hendy et al., 2001). Replacement of inorganic Zn with organic Zn (Zn nicotinate) at 6, 9 and 12 ppm level (Nagalakshmi et al., 2015) and dietary Zn (ZnCO<sub>3</sub>) with Zn-Met at the rates of 50, 75 and 100% in female Sprague Dawley rats (Nagalakshmi et al., 2016) showed no significant change in serum glucose and total protein level. There was significant variation in the activities of ALT in this study among Zn deficient group (T1) and supplemented groups (T2, T3, T4 and T5), therefore, a decreased level of this enzyme indicated no destruction of liver cells. Similar findings of decreased ALP activity were reported in Zn deficient group (Yousef et al., 2002). The Zn deficiency reduced ALP activity in RBC and plasma of rats

(Cho et al., 2007; Lina et al., 2009). Zinc was supplemented @ 0, 30, 60, 90 and 180 mg/kg through Zn-Gly in Sprague-Dawley rat diets and ALP activity increased linearly with supplemental Zn levels (Huang et al., 2016). However, there was no effect of 100% replacement of ZnCO<sub>3</sub> with various sources of organic Zn (Zn-Met, Zn proteinate or Zn propionate) on serum biochemical constituents in rats (Nagalakshmi et al., 2014). In this experiment, Zn supplementation either in inorganic or nano form reduced cholesterol and triglyceride level in male Wistar rats (Paul et al., 2001). Elevated levels of triglyceride were recorded due to Zn deficiency (Eder and Kirchgessner, 1995). The reduced levels of serum cholesterol due to organic Zn supplementation (6-12 ppm level) have been reported (Nagalakshmi et al., 2016).

Table 4. Effect of supplementation of different levels and sources of nano Zn on biochemical parameters in male Wistar rats

| Parameter                 | Group                     |                           |                           |                           |                            |
|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|----------------------------|
|                           | T1                        | T2                        | T3                        | T4                        | T5                         |
| Glucose (mg/dL)           | 57.51 <sup>b</sup> ±1.23  | 80.76 <sup>a</sup> ±1.15  | 79.34 <sup>a</sup> ±1.03  | 81.73 <sup>a</sup> ±0.99  | 83.51 <sup>a</sup> ±0.92   |
| Total protein (g/dL)      | 7.37 <sup>b</sup> ±0.14   | 8.08 <sup>ab</sup> ±0.22  | 7.77 <sup>ab</sup> ±0.21  | 7.91 <sup>ab</sup> ±0.11  | 8.12 <sup>a</sup> ±0.14    |
| Albumin (g/dL)            | 3.67 <sup>c</sup> ±0.09   | 4.30 <sup>ab</sup> ±0.09  | 4.21 <sup>ab</sup> ±0.06  | 3.99 <sup>bc</sup> ±0.07  | 4.54 <sup>a</sup> ±0.17    |
| Globulin (g/dL)           | 3.70 ± 0.20               | 3.78 ± 0.29               | 3.56 ± 0.18               | 3.92 ± 0.18               | 3.59±0.29                  |
| A: G ratio                | 1.01 ± 0.08               | 1.17 ± 0.10               | 1.20 ± 0.06               | 1.03 ± 0.05               | 1.33±0.16                  |
| ALP (U/L)                 | 93.10 <sup>b</sup> ± 0.65 | 99.65 <sup>a</sup> ± 1.40 | 98.87 <sup>a</sup> ± 0.44 | 98.25 <sup>a</sup> ± 1.52 | 100.30 <sup>a</sup> ± 1.35 |
| AST (U/L)                 | 84.07 <sup>a</sup> ± 0.34 | 71.14 <sup>c</sup> ± 0.35 | 73.50 <sup>b</sup> ± 0.51 | 61.20 <sup>d</sup> ± 0.48 | 62.06 <sup>d</sup> ± 0.43  |
| ALT (U/L)                 | 23.82 <sup>a</sup> ± 0.33 | 17.54 <sup>b</sup> ± 0.17 | 18.27 <sup>b</sup> ± 0.28 | 17.42 <sup>b</sup> ± 0.13 | 17.25 <sup>b</sup> ± 0.16  |
| Total cholesterol (mg/dL) | 85.35 <sup>a</sup> ±0.78  | 80.09 <sup>b</sup> ±1.09  | 70.24 <sup>c</sup> ±0.90  | 68.78 <sup>c</sup> ±0.70  | 67.47 <sup>c</sup> ±0.58   |
| Triglyceride (mg/dL)      | 119.60 <sup>a</sup> ±5.45 | 102.43 <sup>b</sup> ±0.91 | 101.10 <sup>b</sup> ±1.64 | 98.24 <sup>b</sup> ±2.48  | 99.62 <sup>b</sup> ±1.24   |

<sup>ab</sup> Mean values bearing different superscripts in a row differ significantly (P<0.05)

## CONCLUSIONS

The feed intake was not affected either by 12 ppm of inorganic Zn or by 3, 6 and 12 ppm of Nano Zn supplementation. However, supplementation of 3 ppm nano Zn was comparable to the groups given either 12 ppm inorganic Zn or 6 and 12 ppm nano Zn in terms of growth performance and blood biochemical attributes in male Wistar rats.

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