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Effects of Dietary Supplementation of Different Levels of Zinc, Manganese and Copper Nanoparticles on Growth Performance and Bioavailability in Commercial Broilers Chicks

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ABSTRACT

Background: Nanotechnology is an emerging field and it has shown the great impact in broiler sector for production. Although extensive research is needed to evaluate the safety of nanominerals.

Methods: A biological study was carried to evaluate the effect of dietary supplementation of inorganic, organic and nano forms of zinc, manganese and copper trace minerals on growth performance and immune status of commercial broiler chicken out for 35 days with 150 numbers of commercial broiler chicken and randomly distributed in 5 treatment groups *viz.,* T_1 (Inorganic 100%), T_2 (Organic 100%), T_3 (Nano 100%), T_4 (Nano 50%) and T_5 (Nano 25%) comprising of 30 birds in each group.

Result: The results revealed that body weight, cumulative body weight gain and feed intake of broiler chicken increased significantly ($p<0.01$) in the 50 and 100% nano trace mineral supplemented groups when compared to other groups without any significant change in FCR. T_3 and T_4 exhibited significant ($p<0.05$) improvement in serum *superoxide dismutase* activity. The mRNA expressions of biomarker gene metallothionein, manganese-*superoxide dismutase* and ceruloplasmin were upregulated in T_3 and T_4 groups revealing that the nano trace mineral supplementation had better bioavailability.

Keywords: Bioavailability, Broiler chicken, Growth performance, Manganese and copper, Nano zinc.

INTRODUCTION

Minerals are one of the most important nutrients in the body. The essential minerals are classified into major elements and trace elements depending upon their concentration in the animal body or amounts required in the diet. Some of the essential trace minerals required by broiler chicken are manganese (Mn), iron (Fe), copper (Cu), iodine (I), zinc (Zn), fluorine (F), cobalt (Co) and selenium (Se). These trace minerals account for only 0.3% of the total body mineral concentration (McDowell, 2003). Trace minerals are typically utilised in trace amounts in animals, although difficulties such as decreased bioavailability, antagonistic interactions and higher bodily excretion rates restrict their effectiveness.

Traditionally, trace minerals have been supplemented in animal diets using inorganic salts such as zinc oxide, manganese oxide or sulphate salts and copper sulphate. However, in recent years there are increased concerns about the usage of inorganic trace mineral salts due to their poor bioavailability and potential to cause mineral antagonism, which impairs absorption of minerals and increases environment pollution. Though the organic forms of minerals can be used in smaller amounts than inorganic forms, the higher cost of using organic minerals still discourages from using them.

Nanotechnology is a rapidly evolving science that makes use of materials with dimensions ranging from 1 to 100 nanometres, resulting in novel properties and applications. Nanoparticles, due to their high physical reactivity, can be used as an alternative promoter of health and growth of broilers in much lesser amounts than inorganic forms of these elements, thereby significantly decreasing the excretion of these minerals into the environment. Zinc is a vital trace mineral that is essential for normal metabolism (King, 2011). Manganese is a critical component of several enzymes involved in the defence system against free radicals, protein metabolism and bone formation in the form of superoxide dismutase, ligase, transferase and hydrolases (Keen *et al*., 2000). Copper is involved in cellular respiration, energy production, collagen synthesis, trace mineral uptake and utilisation, antioxidant activity, heart function, bone formation, tissue keratinization and pigmentation and spinal cord myelination (McDowell, 1992).

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MATERIALS AND METHODS

Biological trial and experimental design

A total of 150-day-old broiler chicks (Cobb 430) were randomly allocated in a completely randomized design with 5 treatments, each of which has 3 replicates of 10 birds each. Biological trial was conducted for a period of 35 days. The dietary treatments consists of a maize-soyabean meal based diet supplemented with inorganic forms of zinc, manganese and copper at 100% of the requirement as per Bureau of Indian Standards (BIS, 2007) was taken as control (T_1), a maize- soyabean meal based diet supplemented with organic forms of zinc, manganese and copper at 100 % of the requirement level (T_2), a maize- soyabean meal based diet supplemented with nano forms of zinc, manganese and copper at 100% of the requirement level (T_3), a maize-soyabean meal based diet supplemented with nano forms of zinc, manganese and copper at 50% of the requirement level (T_4), a maize- soyabean meal based diet supplemented with nano forms of zinc, manganese and copper at 25% of the requirement level (T_5).

Growth performances

Body weights of individual birds were measured early morning before offering feed on days 0, 7, 14, 21, 28 and 35. Cumulative weekly body weight was calculated from the body weight of birds were recorded on day zero and once in every week up to five weeks. Cumulative weekly body weight gain was calculated by subtracting the initial body weight of birds recorded on day one from the cumulative weekly body weight. Cumulative weekly feed intake was calculated by subtracting the quantity of residual feed from feed offered initially. Feed conversion ratio (FCR) was calculated by dividing the total feed consumed in a particular period by body weight gain during that period**.** Mortality of birds was recorded during the experimental period of five weeks and the per cent livability was calculated.

Serum antibody titers against NDV and IBDV

Blood samples were collected from 30 birds (six per treatment) on day 21 and 35 of feeding trial. The serum samples were separated. The concentrations of antibodies were analysed by ELISA using IDEXX IBDV Ab Test and IDEXX NDV Ab Test kits, according to the manufacturer's (IDEXX laboratories, USA) instructions as described by Keck *et al.* (1993).

Superoxide dismutase activity in serum

Serum *superoxide dismutase* activity was measured by the method described by McCord and Fridovich (1969) based on the ability of SOD to inhibit the autooxidation of pyrogallol at pH 8.2. The reaction rate was measured at 420 nm.

Evaluation of Zinc, Manganese and Copper bioavailability by Real time PCR

Metallothionein, Manganese containing superoxide dismutase (MnSOD) and Ceruloplasmin genes were used as biomarkers for zinc, manganese and copper bioavailability utilization in broiler chicken (Varun *et al*., 2017; Zhang *et al*., 2020). About 25 mg of liver and muscle tissue samples were homogenized and treated with 750 µl Trizol Reagent (Sigma) to extract the RNA as described by Kaiser *et al.* (2006). The High-Capacity cDNA Reverse Transcription Kit (Takara Kit) was used for synthesis of cDNA from RNA as per the manufacturer's instructions The cDNA synthesis cycle conditions were 37°C for 15 minutes, followed by 85°C for 5 seconds. The synthesized cDNA was stored at -20°C until further use. Real-time PCR analysis was performed for the expression of genes (Metallothionein, MnSOD and Ceruloplasmin) and β-actin gene was used as the housekeeping gene. Primers used for Real Time PCR are presented in Table 1. The data were analyzed by the $2^{-\Delta\Delta Ct}$ method and normalized using the expression level of the housekeeping gene. Gene expression was measured by 7500 Fast Real Time PCR (Applied Biosystem Inc, CA, USA) with SYBR PREMIX ex TAQ™ (Perfect Real Time, Takara, Shiga, Japan). The thermal cyclic condition used in Real Time PCR was as follows: 95°C for 3 minutes, 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 45 seconds.

Statistical analysis

The data obtained from various parameters were grouped and subjected to statistical analysis through the procedure one way Analysis of Variance (ANOVA) using statistical analysis software (IBM SPSS version 22.0 for windows) as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Growth performance

The results of the study indicated that body weight (Fig 1), cumulative body weight gain (Fig 2) and feed intake (Fig 3)

Table 1: The sense and antisense primer sequences of target genes and reference gene.

Minerals	Genes	Orientation	Primers sequences (5′-3′)	Amplicon size
	β- actin	Forward	GAGAAATTGTGCGTGACATCA	152 bp
		Reverse	CCTGAACCTCTCATTGCCA	
Zinc	*Metallothionein*	Forward	GCAACAACTGTGCCAAGGGC	136 bp
		Reverse	TTTCGTGGTCCCTGTCACCC	
Manganese	*MnSOD*	Forward	TTCCTGACCTGCCTTACGACTAT	138 bp
		Reverse	CCAGCGCCTCTTTGTATTTCT	
Copper	*Cerruloplasmin*	Forward	TGTGGATCCATCCCTGTATTTG	96 bp
		Reverse	TCGAATTCCCATGTCCTGTTAG	

of broiler chicken increased significantly ($p<0.01$) in the 50% nano group (T_4) when compared to other groups. Body weight gain in birds fed diets supplemented with zinc, manganese and copper in nano forms at 50% requirement level was increased around 10.21% when compared to control group (100% inorganic group).

Although 100% organic group (T_2) and 100% nano group (T_3) showed lesser growth and feed intake than the 50% nano group, these groups performed better when compared to 100% inorganic group (T_1) and 25% nano group (T_5). However, body weight gain in 100% organic group and 100% nano group were increased by 4.11% and 4.92%, respectively when compared to control group. Although the inclusion rate of Zn, Mn and Cu is reduced in 25% nano group, there was no significant difference between 25% nano group and 100% inorganic group in terms of growth performance. However, FCR and per cent livability did not vary significantly between the groups throughout the experimental period (Fig 4). Improvement in growth performance in T_4 (50% Nano) over other groups could be

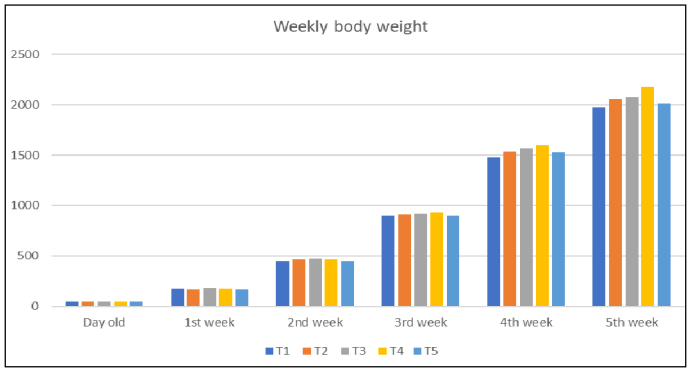


Fig 1: Effect of dietary supplementation of different forms of zinc, manganese and copper on weekly body weight (g) from 0 to 5 weeks of age in commercial broiler chicken (Mean#±S.E.).

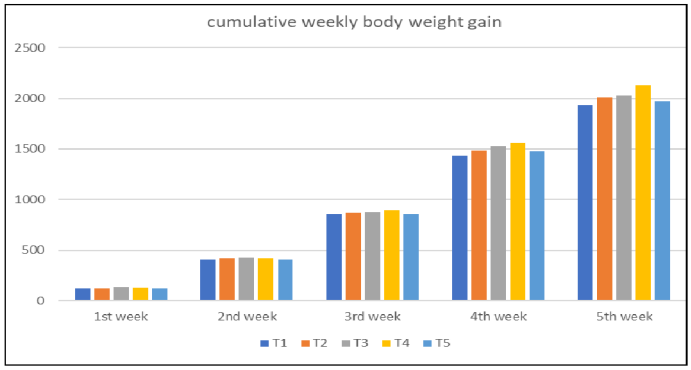


Fig 2: Effect of dietary supplementation of different forms of zinc, manganese and copper on cumulative weekly body weight gain (g) from 0 to 5 weeks of age in commercial broiler chicken (Mean#±S.E.).

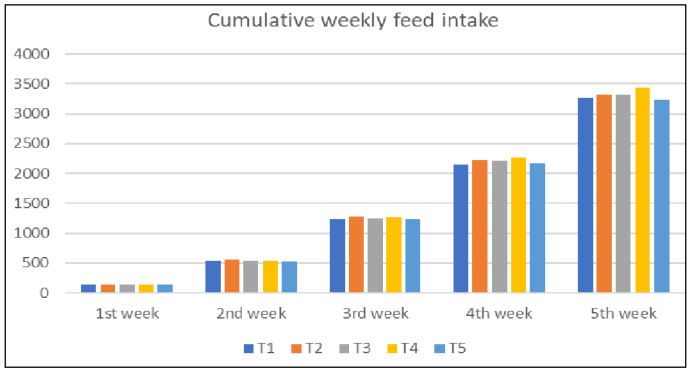


Fig 3: Effect of dietary supplementation of different forms of zinc, manganese and copper on cumulative weekly feed intake (g) from 0 to 5 weeks of age in commercial broiler chicken (Mean#±S.E.).

attributed to higher bioavailability of Zn, Mn and Cu at reduced inclusion levels and absence of negative interaction among minerals in GI tract. Further, on par growth performance recorded in T_5 (25% Nano) compared to control group (T_1-100% inorganic) might be due to the better bioavailability of nano minerals compared to their respective mega particles.

Similar results were observed by the other researchers and they reported that supplementation of nano forms of Zn, Mn and Cu resulted in higher body weight gain and feed intake in commercial broiler chicken when compared to their respective inorganic forms and organic forms (El-Katcha *et al*., 2017; Alkhtib *et al*., 2020; Matuszewski *et al*., 2020). They also reported that there was no significant change in FCR.

The higher body weight gain observed in nano Zn, Mn and Cu supplemented groups might be due to increased absorption of nano Zn, Mn and Cu from GI tract. Because of smaller particle sizes of Zn, Mn and Cu nanoparticles, a faster diffusion might have occurred in GI tract to reach the intestinal lining. Nano particles with reduced size have greater surface area, thereby they exhibit a higher rate of absorption in the gastrointestinal tract (Hussain *et al*., 2001). Nano-sized minerals distribute quickly from the circulation to the tissues and they are mostly targeted at organs with a well-perfused reticuloendothelial system (RES), such as the spleen and liver (Geraets *et al.,* 2014).

Serum antibody titers against NDV and IBDV

The results of the present study indicated that there was no significant difference between the treatment groups in antibody titer value against NDV after 14th day of vaccination. After 28th day of vaccination, the control groups had significantly higher titer values against NDV when compared to all the other groups (Fig 5).

The results of the present study were in accordance with Sabaghi *et al*. (2021) who found that the nano manganese supplementation at 100 ppm and 150 ppm levels in broiler chicken showed reduced ($p<0.05$) antibody titer values against NDV when compared to the inorganic manganese supplementation.

Superoxide dismutase activity in serum

Superoxide dismutase activity in broiler chicken is influenced by the supplementation of different forms of Zn, Mn and Cu. In the present study, a significant increase in serum SOD activity was observed in 100% nano (T_3) and 50% nano (T_4) groups when compared to other treatment groups. However, no difference was observed between 100% inorganic group (T_1) and 25% nano group (T_5) (Fig 6).

Similar results of increased SOD enzyme activity in plasma were reported by Abedini *et al*. (2018) with the ZnO nanoparticle supplementation in broilers when compared to the control group. El-Kazaz and Hafez, (2020) concluded

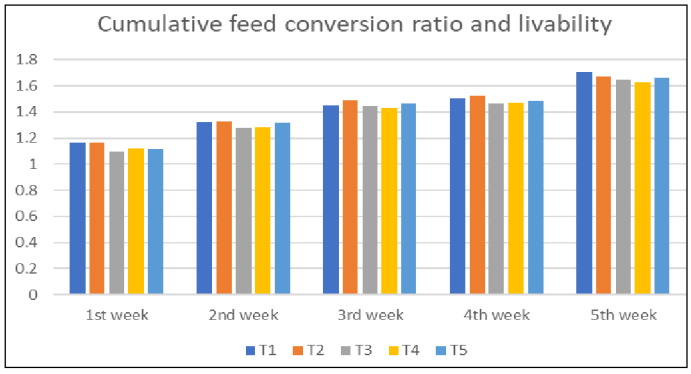


Fig 4: Effect of dietary supplementation of different forms of zinc, manganese and copper on cumulative feed conversion ratio and livability (%) from 0 to 5 weeks of age in commercial broiler chicken.

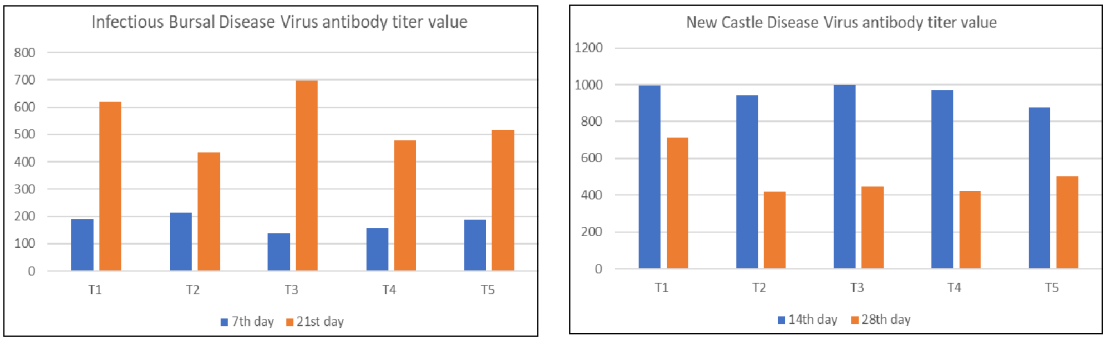


Fig 5: Effect of dietary supplementation of different forms of zinc, manganese and copper on serum antibody titer value against NDV and IBDV in commercial broiler chicken (Mean$^{\#}$±S.E.).

that broilers fed with copper nanoparticles have considerable increase ($p<0.05$) in serum copper and SOD levels compared to $CuSO_4$ treated birds. It was reported that Zn, Mn and Cu nanoparticles were found to have higher bio-availability than their inorganic and organic counterparts because of their faster rate of absorption (Jankowski *et al*., 2020).

Bioavailability of biomarkers of zinc, manganese and copper

The results of the present study indicated that the metallothionein gene expression in liver and muscle was higher in 100 and 50 % nano mineral supplemented groups when compared to other treatment groups (Fig 7). Similarly, Varun *et al*. (2017) reported that the broiler chicken fed with nano zinc had relatively higher expression of metallothionein gene in liver when compared to inorganic zinc.

MnSOD mRNA levels in both liver and muscle were higher ($p<0.01$) in 100% and 50% nano mineral supplemented groups when compared to inorganic mineral supplemented group. Similarly, Zhang *et al*. (2020) reported that the dietary supplementation of manganese improved the gene expression of MnSOD thereby increasing the intestinal immunity in broiler chicken.

Liver ceruloplasmin mRNA levels were not influenced by dietary copper supplementation of inorganic, organic or nano copper in broilers. Accordingly, Scott *et al.* (2018) reported that ceruloplasmin mRNA gene expression was not affected by dietary source of copper in liver tissue of

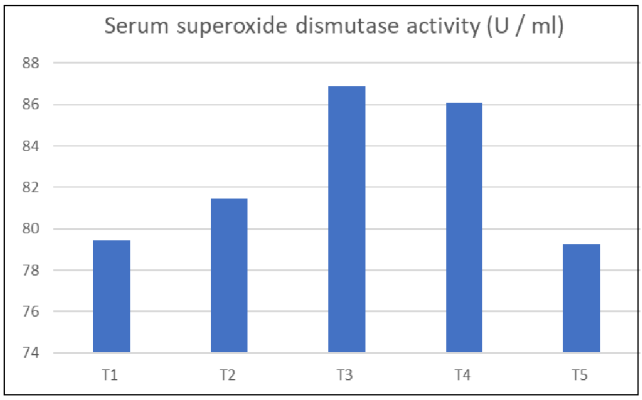
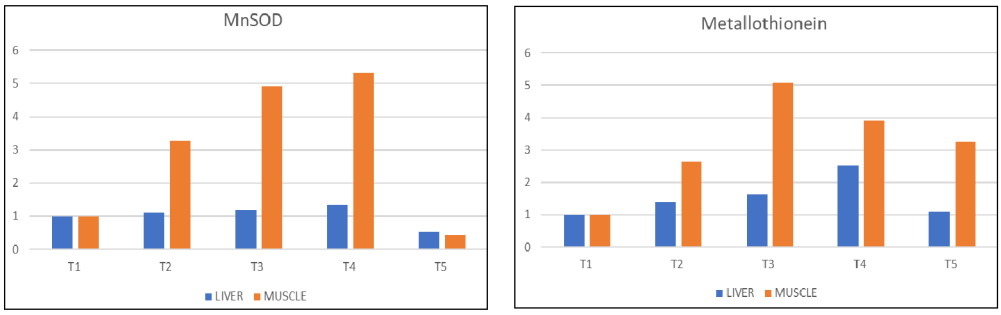
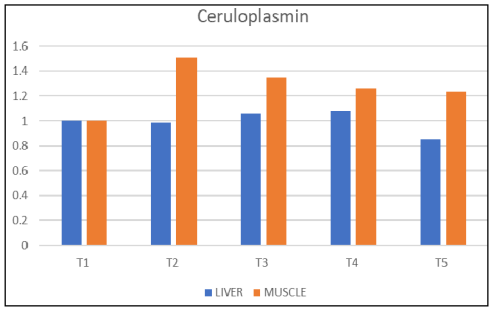


Fig 6: Effect of dietary supplementation of different forms of zinc, manganese and copper on serum *superoxide dismutase* activity on 35th day of age in commercial broiler chicken.



(a) **(b)**



(c)

Fig 7: Effect of dietary supplementation of nano forms of zinc, manganese and copper on their relative gene expression in liver and muscle using real time PCR.

broiler chicken. Hence, liver ceruloplasmin mRNA expression might not be an efficient biomarker to indicate copper bioavailability. However, significant upregulation was observed in muscle ceruloplasmin mRNA expression when the birds are supplemented with organic and nano forms of copper at 100 and 50% requirement levels.

It was revealed from this study that the supplementation of nano forms of zinc and manganese upregulated the expression of metallothionein and MnSOD in liver and muscle tissue. Whereas, nano supplementation of copper upregulated the expression of ceruloplasmin in muscle tissue. These upregulation of biomarkers for zinc, manganese and copper indicated that the nano form of supplementation had increased the bio-availability of these three trace minerals in broiler chicken.

CONCLUSION

The supplementation of nano zinc, manganese and copper at 25% requirement level was sufficient to raise the broiler chicken without any deficiency symptoms. The growth performance was at par with the birds fed with diets supplemented with inorganic forms of zinc, manganese and copper at 100% requirement level. However, improved growth performance was observed in nano zinc, manganese and copper supplemented group at 50% requirement level.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Animal welfare statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. The standards of care used in the study was approved by Institutional Animal Ethics Committee (Approval Lr. No. 08/SA/IAEC/2021).

Conflict of interest: None.

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