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Effect of Biosynthesised Nano-zinc on Blood Biochemical Profile, Antioxidant Status and Immune Response in Vanaraja Birds

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ABSTRACT

The experiment was conducted during December, 2021 to February, 2022 at the location of research was poultry farm, directorate of poultry research Hyderabad, Telangana, India to investigate the efficacy of biosynthesized nano-zinc as a substitute for traditional zinc salt in the diet of Vanaraja chickens, focusing on its impact on blood biochemical profile, antioxidant status, and immune response. A total of 324-day-old Vanaraja chicks were randomly assigned to six dietary treatments, including a negative control (no supplemental zinc), a basal diet supplemented with zinc oxide (60 mg Zn kg^{-1}), and four groups receiving biosynthesized nano-zinc at levels of 15, 30, 45, and 60 mg Zn kg^{-1} diet. The trial spanned 56 days with 9 replicates per treatment group. Results demonstrated that nano-zinc supplementation at 30 mg kg^{-1} significantly ($p<0.05$) improved humoral immunity compared to the control, matching the immune response achieved with 60 mg kg^{-1} zinc oxide. No adverse effects on serum enzyme profiles or biochemical parameters were observed across treatments, indicating the safety of nano-zinc as a feed additive. From the current study, it may be concluded that the conventional zinc oxide can be replaced with nanoparticle forms at a reduced level without any adverse effect on health assessed through serum biochemical profile. In order to recommend the level of dose reduction, more studies with different breeds, indifferent agro-climatic zones during different seasons are recommended.

KEYWORDS: Antioxidant status, immunity, nano-zinc, vanaraja

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1. INTRODUCTION

The poultry industry is a significant contributor to the global food supply, providing an affordable and high-quality source of protein. However, maintaining the health and productivity of poultry birds is a continual challenge, necessitating the exploration of new and innovative solutions. In recent years, the use of nanoparticles, particularly zinc nanoparticles, has emerged as a promising strategy to enhance poultry health and performance due to their unique properties and bioactivities (Abedini et al., 2017). Zinc is an essential trace mineral that plays a critical role in various physiological processes, including enzyme function, protein synthesis, and immune response (Prasad, 2014). Traditional zinc supplementation in poultry diets often faces issues related to bioavailability and potential environmental contamination from excreted unabsorbed zinc (Maret and Sandstead, 2006). To address these challenges, biosynthesized zinc nanoparticles (ZnNPs) have garnered significant attention. The nanoscale size and increased surface area of ZnNPs can potentially enhance their bioavailability and biological activity, offering a more efficient means of zinc supplementation (Sahoo et al., 2014). Biosynthesis of ZnNPs using plant extracts is a green and sustainable approach, leveraging the reducing and stabilizing properties of phytochemicals present in plants (Singh et al., 2016; Kharissova et al., 2013). Neem (Azadirachta indica) is particularly notable for its rich phytochemical profile, including flavonoids, terpenoids, and other antioxidants, which facilitate the biosynthesis of stable ZnNPs (Siddiqui et al., 2012). These biosynthesized ZnNPs have shown promise in various biomedical and agricultural applications due to their antimicrobial, antioxidant, and immunomodulatory (Puvvada et al., 2019). Vanaraja birds, a popular dual-purpose breed in India known for their high meat and egg production, can benefit significantly from optimized nutritional strategies, including enhanced zinc supplementation. However, comprehensive studies on the impact of ZnNPs on the blood biochemical profile, antioxidant status, and immune response in Vanaraja birds remain limited. This study aims to fill this gap by evaluating the effects of biosynthesized nano-zinc on these parameters in Vanaraja birds. Previous research has demonstrated that nanoparticles can modulate various physiological and biochemical pathways in poultry (Fathi et al., 2016; Khan et al., 2015; Mohammadi et al., 2015). For instance, dietary supplementation with zinc oxide nanoparticles has been shown to improve growth performance, enhance immune response, and boost antioxidant enzyme activities in broilers (Ahmadi et al., 2013; Swain et al., 2016). The antioxidant properties of ZnNPs are of particular interest, given the role of oxidative stress in impairing poultry health and performance (Gao et al., 2017; Ebrahimzadeh et al., 2016). Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defenses, leading to cellular damage and inflammation (Surai, 2016). ZnNPs, with their inherent antioxidant capabilities, can help mitigate oxidative stress, thereby promoting better health and immune function in poultry (Rizwan et al., 2017; Ashouri et al., 2021). Furthermore, the immune-modulatory effects of ZnNPs are crucial for enhancing disease resistance in poultry. Zinc is known to influence various aspects of the immune system, including the function of immune cells and the production of cytokines (Kidd, 2004). Studies have indicated that ZnNPs can enhance the immune response in poultry, potentially leading to improved disease resistance and reduced reliance on antibiotics (Sahoo et al., 2014; Rajendran et al., 2020). In conclusion, the biosynthesis of nano-zinc using neem leaf extracts offers a novel and sustainable approach to zinc supplementation in poultry diets. This study aims to investigate the effects of these ZnNPs on the blood biochemical profile, antioxidant status, and immune response in Vanaraja birds, providing valuable insights into their potential benefits and mechanisms of action. This study aimed to investigate the effects of biosynthesized nano-zinc, compared to inorganic copper sulfate, on immune status, blood biochemical profile, and antioxidant status in Vanaraja chickens.

2. MATERIALS AND METHODS

The place experiment was, ICAR-Directorate of Poultry Research, Hyderabad, Telangana, India. The experiment was conducted between December 2021 to February 2022. All the procedures required for this experiment were in accordance with the guidelines of animal ethics committee of the institution. Day old Vanaraja chicks were selected (n=324) which had a mean body weight of 37.35+0.14. They were randomly assigned one of the six dietary treatment, each treatment having nine replicates. They were wing banded and housed in three tire battery brooders. The groups were basal diet without any supplemental zinc (control, Zn_0), basal diet supplemented with 60 mg Zn kg^{-1} diet from zinc oxide (Zn_60) and the remaining four groups were fed with nano-zinc at 15, 30, 45 and 60 mg Zn per kg diet (ZnNP_15, ZnNP_30, ZnNP_45 and ZnNP_60) respectively. Diets were formulated to meet nutrient requirements of chicken as per Anonymous (1992) for all nutrients except zinc. Zinc was added separately as per the treatment levels. Nano-zinc powder was hand-mixed using standard mixing technique. The nano-zinc was synthesized using leaf extracts of Azadirachta indica (Neem leaf) as per the procedure standardized by Bhuyan et al. (2015). Formation of nano particles were checked using particle size analysis and transmission electron microscopy

(TEM) and ensured that particles were below 100 nm. The ingredient composition and nutrient composition of chick feed and grower feed are presented in the Table 1. Zinc content of different treatment feeds is presented in Table 2.

The chicks were brooded by providing supplementary heat till 4 weeks of age using incandescent bulbs. The chicks were vaccinated against Newcastle and Infectious bursal diseases as per the the standard vaccination schedule. For collection of serum, about 2–3 ml blood was collected through the brachial vein from one chick in each replicate (9 chicks treatment^{-1}) into non-heparinised tubes on 56th day of experiment, about two to three ml of blood was collected. Serum was separated for estimation of biochemical parameters (glucose, total protein, albumin, cholesterol and triglycerides, blood urea nitrogen) and enzymes by using reagent kits (Jeev Diagnostics Pvt. Ltd. Chennai, India & ERBA Diagnostics, Mumbai, India). Antioxidant enzymes of the serum viz. glutathione peroxidase, glutathione reductase and serum enzymes were estimated as per the procedures. The humoral immunity status was assessed by antibody titres against Newcastle disease. In order to get antibody titres on 56th day, an additional vaccination of R2B strain was given to the experimental birds in different groups on 42nd day of age for the study. Antibody titres in sera against ND virus were measured by haemagglutination test. For measuring cell-mediated immune response in the birds, Phyto-haemagglutinin type P (PHA-P) was used as antigen. The test was conducted at the age of 30 days as per the method of Cheng and Lamont (1988), by injecting the solution of PHA-P antigen in of 1 mg/ml in sterile phosphate buffer saline, (PBS) intradermal in inter-digital space between 3rd and 4th toe of the right foot of the individual chick. The Swelling was measured to calculate Foot web index (FWI) as the cell mediated immune response of the bird. One bird per replicate near to the mean body weight of the respective group were selected on 56th day for sacrifice to assess the immunity status as per the size of bursa of Fabricius. The data obtained from different treatments were pooled replicate wise. The data were subjected to one way analysis of variance (ANOVA) (Snedecor and Cochran, 1994) in the Statistical Package for Social Sciences software Anonymous (2012). Whenever significant differences were found (at $p<0.05$), the treatment means were compared using Tukey's test. The results were expressed as mean and standard error of mean.

Table 1: Ingredient and chemical composition of basal diets

Ingredient (kg)	Chick diet (0–3 Week)	Grower diet (4–8 week)
Maize	58.07	65.54
Soybean meal	37.32	29.85
Stone grit	1.60	1.6
Dicalcium phosphate	1.90	1.90
Common Salt	0.35	0.35
Sodium bicarbonate	0.10	0.10
DL-Methionine	0.19	0.19
Lysine	0.04	0.04
Trace mineral mix (without copper)*	0.10	0.10
Vitamin ADK pre mix**	0.015	0.015
Vitamin B-Complex mix***	0.015	0.015
Choline chloride	0.10	0.10
Toxin binder	0.10	0.10
Tylosine	0.05	0.05
Coccidiostat	0.05	0.05
Total	100.00	100.00
Chemical composition		
ME (kcal kg^{-1})^1	2803	2881
Crude protein (%)	22.45	20.12
Ether extract (%)	1.78	1.92
Crude fibre (%)	3.12	3.26
Calcium (%)	1.02	1.03
Available phosphorus (%)	0.45	0.42
Lysine^1	1.36	1.28
Methionine^1	0.60	0.52

*Contains Ca-32%, P-9%, Fe-2000 ppm, I-0.01%, Mn-0.4% and Zinc-0.4%; **: Each gram contains Vitamin A-82500 IU, Vitamin B$_2$-50 mg, Vitamin D$_3$-12000 IU and Vitamin K-10 mg. ***: Each gram contains Vitamin B$_1$-4 mg, B$_6$-8 mg, B$_{12}$-40 mg, E-40 mg, Calcium pantothenate-40 mg, Niacin-60 mg; Calculated value

Table 2: Zinc concentration in different dietary treatments

Treatment	Zinc source	Zinc added (mg kg^{-1})	Analysed zinc concentration# (mg kg^{-1})
Zn_0	-	0	57
Zn_60	Zinc	60	128
ZnNP_15	Biosynthesised nano-zinc	15	74
ZnNP_30	Biosynthesised nano-zinc	30	91
ZnNP_45	Biosynthesised nano-zinc	45	108
ZnNP_60	Biosynthesised nano-zinc	60	129

#Values based on triplicate analysis

3. RESULTS AND DISCUSSION

Effect of nano-zinc on immunity had been presented in the Table 3. The antibody titres ranged between 7.67 and 10.11. The enhancement of antibody titres observed in this study suggests that biosynthesized nano-zinc is more effective than conventional zinc sources in stimulating the humoral immune response. This finding is supported by several studies that have demonstrated the superior bioavailability and immunomodulatory effects of nano-zinc particles. For instance, Swain et al. (2016) reported that nano-zinc oxide enhanced the immune response and growth performance in broiler chickens compared to traditional zinc sources. The increased surface area and reactivity of nano-zinc particles facilitate better absorption and utilization, leading to enhanced immune function. In the study conducted by Hafez et al. (2019), nano-zinc of 40 mg kg^{-1} was found to increase IgY content of serum significantly ($p<0.05$) over the control group receiving equal dose of inorganic zinc. In the current study, there was numerical superiority of antibody titres of nano-zinc fed groups over inorganic zinc without any statistical significance. The current study measured all immunoglobulins compared to specific IgY antibodies by Hafez et al. (2019). In their study antibody titres did not differ significantly between the two levels of nano-zinc (40 and 80 mg kg^{-1}). Their experimental groups did not include any reduced nano-zinc compared to inorganic zinc, whereas in the current investigation, nano-zinc at 25% of inorganic zinc also was statistically similar in stimulating antibody response. Hence it is inferred that the findings of the present study are partially similar to their findings.

The results of the current trial also partially agree with results of the trial conducted by Mao and Lien (2017), the nano-zinc fed group exhibited significantly ($p<0.05$) better serum levels compared to birds receiving equal level of inorganic zinc.

Table 3: Effect of feeding zinc nanoparticles on immunity of Vanaraja chicken

Treatment	Antibody titres	CMI (Ratio)	Weight of Bursa (g)
Zn_0	7.67a	0.38	2.26
Zn_60	9.00ab	0.39	2.53
ZnNP_15	9.33ab	0.57	2.59
ZnNP_30	9.44 b	0.49	2.57
ZnNP_45	9.56b	0.47	2.70
ZnNP_60	10.11b	0.46	2.55
SEm±	0.19	0.03	0.06
p Value	0.005	0.619	0.468

[a,b]Figures within a column bearing different superscripts differ significantly; CMI: Cell mediated immunity

Despite the significant improvements in antibody titres, no significant effects were observed on cell-mediated immunity (CMI) across the different zinc treatments. This is consistent with findings from Sahin et al. (2010), who reported that while zinc supplementation could enhance humoral immunity, its effects on CMI were less pronounced. The CMI is primarily mediated by T-cells, which may have different nutritional requirements or regulatory mechanisms compared to B-cells involved in antibody production. This discrepancy suggests that zinc's role in immune modulation may be more effective in humoral immunity than in cell-mediated responses (Sahin et al., 2010). The lack of significant differences in the weight of the bursa of Fabricius among the treatment groups indicates that zinc supplementation, whether in conventional or nano form, does not adversely affect this lymphoid organ. The bursa is crucial for the development of the avian immune system, and its weight is often used as an indicator of immune status. Our findings are in line with a study by Ognik and Krauze (2012), which showed that dietary zinc supplementation did not significantly alter bursa weight in broiler chickens. This suggests that while nano-zinc can enhance certain aspects of immune function, it does not cause hypertrophy or atrophy of immune organs, maintaining overall physiological balance (Ognik and Krauze, 2012).

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Effect of nano-zinc on the antioxidant parameters of serum is presented in the Table 4. The data indicate that all the tested antioxidant parameters viz., lipid peroxidation per mg protein, glutathione peroxidase activity, glutathione reductase activity and superoxide dismutase activity were all statistically similar among all the treatments.

Lipid peroxidation is a critical indicator of oxidative stress and cellular damage. The LP levels in this study ranged from 0.66 to 0.89 mg protein across all treatments, with ZnNP_15 showing the lowest LP level (0.66 mg protein) compared to the control group (0.83 mg protein). Although

Table 4: Effect of feeding zinc nanoparticles on antioxidant status of Vanaraja chicken

Treatment	LP/mg protein	GPX (U/l)	GRX (U/l)	SOD (U/l)
Zn_0	0.83	1328	3056	5.97
Zn_60	0.81	1294	3133	7.20
ZnNP_15	0.66	1402	3180	4.63
ZnNP_30	0.89	1469	3035	4.39
ZnNP_45	0.86	1470	2978	6.34
ZnNP_60	0.70	1499	2968	6.64
SEm±	0.04	181	74	0.47
p value	0.527	0.859	0.960	0.462

LP/mg protein: Lipid peroxidation per mg of serum protein; GPX: Activity of glutathione peroxidase; GRX: Activity of glutathione reductase; SOD: Activity of superoxide dismutase

the differences were not statistically significant ($p=0.527$), the reduction in LP in the ZnNP_15 group suggests a potential protective effect of lower doses of ZnNP against lipid peroxidation. Similar findings were reported by Selim et al. (2015), who observed that dietary nano-zinc oxide reduced oxidative stress markers in broilers, potentially due to its higher bioavailability and better cellular uptake. Moreover, Jiao et al. (2014) demonstrated that nano-zinc supplementation decreased LP levels in broilers, further supporting the notion that nano-zinc can mitigate oxidative damage. Contradictory results were reported by Zhao et al. (2014) who reported a positive correlation between level of nano-zinc in the diet and the antioxidant activity.

GPX activity, an essential enzyme in the antioxidant defense system, did not show significant differences across treatments ($p=0.859$). However, the highest GPX activity was observed in the ZnNP_60 group (1499 U/l), indicating a possible dose-dependent effect of ZnNP on enhancing GPX activity. This trend aligns with the study by Mirzaei et al. (2015), which found that nano-zinc supplementation improved the antioxidant enzyme activities in broilers, suggesting that ZnNP may enhance the body's enzymatic antioxidant defense.

The GRX activity varied slightly among treatments, with no significant differences observed ($p=0.960$). The highest GRX activity was recorded in the ZnNP_15 group (3180 U/l), while the control group showed a slightly lower activity (3056 U/l). These results are consistent with the findings of Gheisar et al. (2015), who reported that dietary zinc supplementation could modulate the activity of antioxidant enzymes, although the effects might not always be statistically significant.

SOD activity, another crucial antioxidant enzyme, showed no significant variation across the different zinc treatments ($p=0.462$). The control group exhibited a SOD activity of 5.97 U/l, while the ZnNP_15 and ZnNP_30 groups had slightly lower activities (4.63 U/l and 4.39 U/l, respectively). The highest SOD activity was observed in the Zn_60 group (7.20 U/l). These findings are in line with those of Swain et al. (2016), who noted that zinc supplementation could influence SOD activity, although the response may vary depending on the zinc form and dose used. Other investigators, Hafez et al. (2019) also found that superoxide dismutase (SOD) level of both treatment groups of 40 and 80 mg kg^{-1} diet were significantly ($p<0.05$) higher than the control group (80 mg kg^{-1} normal zinc), while both levels of nano-zinc fed groups did not differ significantly. Lee et al., 2021 also reported significant ($p<0.05$) improvement of superoxide dismutase activity in the liver of broilers fed with hot-melt-extruded zinc at 80 mg kg^{-1} (or physically synthesized nanao zinc) compared to birds received inorganic zinc in equal concentration. study by Mirzaei et al. (2015), which found that nano-zinc supplementation improved the antioxidant enzyme activities in broilers, suggesting that ZnNP may enhance the body's enzymatic antioxidant defense.

Majority of investigators concluded that nano-zinc enhances antioxidant status, the present study concluded otherwise. The reason could be that, most the studies used commercial broilers, while the present study used the Vanaraja breed which is a dual purpose, stress resistant bird (Rajkumar et al., 2018). The season of trial (midwinter to late winter) could be another reason for lack of response in terms of antioxidant activity.

The effects of feeding zinc nanoparticles (ZnNP) on the serum biochemical parameters of Vanaraja chickens were examined (Table 5), including glucose, total protein, albumin, globulin, cholesterol, triglycerides, and blood urea nitrogen (BUN) levels. The glucose levels in this study ranged from 124.53 to 164.82 mg dl^{-1} across all treatments. Although the differences were not statistically significant ($p=0.488$), the ZnNP_45 group exhibited the highest glucose level (164.82 mg dl^{-1}). This increase in glucose levels with ZnNP supplementation may suggest an improved energy metabolism in the birds. Similar findings were observed by Mohammadigheisar et al. (2015), who reported elevated glucose levels in broilers supplemented with nano-zinc, indicating enhanced glucose utilization. The total protein, albumin, and globulin levels did not show significant differences among the treatments ($p>0.05$). The total protein ranged from 4.79 to 5.17 g dl^{-1}, albumin from 3.38 to 3.50 g dl^{-1}, and globulin from 1.34 to 1.67 g dl^{-1}. These results suggest that ZnNP supplementation does not significantly affect protein metabolism. This is consistent with findings from Swain et al. (2016), who also

Table 5: Effect of feeding zinc nanoparticles on serum biochemical parameters of vanaraja chicken

Treatment	Glucose (mg dl^{-1})	Total protein (g dl^{-1})	Albumin (g dl^{-1})	Globulin (g dl^{-1})	Cholesterol (mg dl^{-1})	Triglycerides (mg dl^{-1})	BUN (mg dl^{-1})
Zn_0	124.53	4.79	3.45	1.34	160.95	161.31	25.05^{ab}
Zn_60	138.42	5.05	3.44	1.60	150.84	127.55	23.70^{a}
ZnNP_15	154.15	4.91	3.38	1.53	134.66	162.81	24.93^{ab}
ZnNP_30	141.90	5.01	3.43	1.59	144.91	132.69	25.56^{b}
ZnNP_45	164.82	5.16	3.49	1.67	131.66	141.37	25.33^{ab}
ZnNP_60	142.55	5.17	3.50	1.67	145.12	134.83	24.30^{ab}
SEm±	5.85	0.12	0.03	0.10	5.38	7.75	0.19
p Value	0.488	0.945	0.884	0.939	0.697	0.716	0.039

noted no significant changes in these parameters with zinc supplementation in broilers. However, the slight increases observed in the ZnNP_60 group could indicate a trend towards improved protein synthesis and immune function. Cholesterol levels ranged from 131.66 to 160.95 mg dl^{-1}, and triglyceride levels from 127.55 to 162.81 mg dl^{-1}, with no significant differences observed (p=0.697 and p= 0.716, respectively). The lowest cholesterol level was found in the ZnNP_45 group (131.66 mg dl^{-1}), while the control group had the highest (160.95 mg dl^{-1}). Similarly, the Zn_60 group showed the lowest triglyceride level (127.55 mg dl^{-1}). These findings align with those reported by Hongfu and Qingyun (2011), who found that nano-zinc reduced cholesterol levels in broilers, potentially due to its role in lipid metabolism. Conversely, contradictory findings by El-Hack et al. (2017) indicated no significant effect of nano-zinc on cholesterol and triglyceride levels, highlighting the variability in responses based on experimental conditions and bird genetics. The BUN levels showed significant differences among treatments (p=0.039), ranging from 23.70 to 25.56 mg dl^{-1}. The ZnNP_30 group had the highest BUN level (25.56 mg dl^{-1}), while the Zn_60 group had the lowest (23.70 mg dl^{-1}). Elevated BUN levels in the ZnNP_30 group might indicate higher protein catabolism or nitrogen excretion. These results are consistent with the findings of Gheisar et al. (2015), who reported similar trends in BUN levels with nano-zinc supplementation, suggesting an impact on nitrogen metabolism.

Effect of feeding zinc nanoparticles to Vanaraja chicken on serum enzyme content, serum copper, calcium and phosphorus content is presented in the Table 6. The results revealed that lack of significant changes in calcium and phosphorus levels suggests that ZnNPs do not adversely affect the homeostasis of these critical minerals, corroborating findings by Swain et al. (2016) and Kalita et al. (2015). The data indicated no effect of nano copper on the serum enzyme activities of SGOT, SGPT and ALP. These results indicate that nanoparticles of zinc are safe to feed to Vanaraja chicken. These results agreed with (Sawyer et al., 2017; Elnesr et al., 2019), where SGPT, SGOT, and ALP levels indicate that ZnNP supplementation up to 60 ppm does not induce hepatic stress or damage, supporting the safety profile of ZnNPs in poultry nutrition. The results of this study, with respect to serum zinc levels differed from Kumar et al (2020) who reported significantly ($p<0.01$) higher zinc levels in serum due to feeding of hot melt extruded (HME) zinc, form of physically synthesized

Table 6: Effect of feeding zinc nanoparticles on serum enzymes and minerals of vanaraja chicken

Treatment	Zinc (ppm)	Calcium (mg dl^{-1})	Phosphorus (mg dl^{-1})	SGPT (U/l)	SGOT (U/l)	ALP (U/l)
Zn_0	170.19	10.30	5.29	8.92	110.13	341.01
Zn_60	198.14	9.66	5.35	8.53	103.15	373.21
ZnNP_15	190.10	9.84	5.31	6.98	104.31	317.11
ZnNP_30	204.39	10.22	5.39	6.01	115.43	333.50
ZnNP_45	186.52	9.60	5.37	8.34	123.83	321.08
ZnNP_60	200.81	10.18	5.14	8.14	101.47	319.39
SEm±	6.10	0.19	0.13	0.73	6.40	23.35
p Value	0.658	0.862	0.996	0.881	0.923	0.988

nano-zinc. This difference was observed at nano-zinc levels of 75 and 100 ppm compared with 25 or 50 ppm, which was higher than the maximum level of nano-zinc in the current experiment i.e. 60 ppm. In terms of serum zinc, calcium and phosphorus, the current study results differed with the findings of Feng et al (2010) who reported that higher dietary zinc causes significantly ($p<0.05$) higher zinc, calcium and phosphorus in the serum.

In the current study, lack of significant effect in negative control group and where diet is devoid of supplemental zinc, *vis-à-vis* other groups could be due to naturally occurring zinc of 57 mg kg^{-1} (Table 2) which is almost equal to the recommended dose of 60 mg kg^{-1} diet. The experimental birds were also a slow growing breed, Vanaraja which takes 56 days to reach body weight around 2 kg, while in the other studies it was commercial broilers, where similar body weight is achieved by 40th day of age.

4. CONCLUSION

Replacing traditional zinc with zinc nanoparticles affected the serum biochemical parameters, antioxidant status, and immunity. Nano-zinc at a reduced level of 30 mg kg^{-1} diet was as effective as inorganic zinc at 60 mg kg^{-1} diet in terms of humoral immunity. Nano-zinc did not impact cell-mediated immunity or the size of the bursa of Fabricius. Dietary zinc levels could be reduced by replacing traditional zinc sources with biosynthesized nano-zinc.

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