



Effect of Biosynthesized Nano Zinc on Growth Performance, Nutrient Utilization and Tissue Mineral Concentration in Vanaraja Chicken

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ABSTRACT

This study evaluated the effects of feeding biosynthesized zinc nanoparticles (ZnNPs) on growth performance, nutrient utilization, and tissue mineral concentrations in Vanaraja chickens. Birds were divided into six groups: a zinc-negative control, a positive control receiving 60 ppm inorganic zinc, and four groups supplemented with 15 ppm, 30 ppm, 45 ppm, and 60 ppm ZnNPs. Over an 8-week period, parameters such as feed consumption, growth rates, feed conversion ratio (FCR), nutrient utilization, zinc bioavailability, and tissue zinc concentrations were recorded. Feed intake during the starter phase remained stable, averaging 461.96 to 472.58 g, while significant decreases were observed in the finisher phase (2320.65 to 2447.93 g), indicating enhanced feed efficiency associated with ZnNPs. Growth rates improved significantly during the finisher phase (953.11 to 1036.06 g) and overall (1162.21 to 1246.67 g), highlighting the potential of ZnNPs to optimize nutrient absorption. FCR values ranged from 2.24 to 2.51, with ZnNP groups achieving efficiencies comparable to the positive control, suggesting that ZnNPs can replace conventional zinc sources. Although nutrient utilization effects were statistically insignificant, higher ZnNP doses increased zinc retention in bone (up to 347.28 ppm) and liver tissues. These findings indicate that ZnNP supplementation can enhance growth efficiency and zinc bioavailability in poultry.

Key Words: Bioavailability, Bone Zinc, FCR, Growth, Mineral Retention, Poultry

INTRODUCTION

Zinc supplementation is achieved using inorganic zinc sources, such as zinc oxide and zinc sulphate. However, recent advancements in nanotechnology have introduced biosynthesized nano zinc particles as a potential alternative due to their enhanced bioavailability and unique physicochemical properties (Khan *et al*, 2020; Rani *et al*, 2018). Nano zinc particles, which range in size from 1 to 100 nano meters, have shown to offer improved absorption and utilization compared to their conventional counterparts (Wang *et al*, 2019; Zhang *et al*, 2020). In poultry

nutrition, nano zinc has been investigated for its effects on growth performance, nutrient utilization, and tissue mineral concentrations. Several studies have reported that nano zinc supplementation can lead to significant improvements in body weight gain and feed conversion ratio (FCR) in broilers (Ibrahim *et al*, 2017) and layers (Saleh *et al*, 2018). For instance, Mohammadi *et al* (2015) observed that nano zinc at specific dosages significantly enhanced growth parameters compared to conventional zinc sources. Similarly, Bami *et al* (2018) found that nano zinc supplementation led to improved feed

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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)	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 B₂-50 mg, Vitamin D₃-12000 IU and Vitamin K-10 mg. * Each gram contains Vitamin B₁-4 mg, B₆-8 mg, B₁₂-40 mg, E-40 mg, Calcium pantothenate-40 mg, Niacin-60 mg.

efficiency in broilers. The bioavailability of zinc is crucial for its effectiveness in poultry diets. Research has demonstrated that nano zinc particles were more readily absorbed and utilized by poultry compared to larger zinc particles or inorganic zinc compounds (Asheer *et al*, 2018; Zhao *et al*, 2014). This enhanced bioavailability has been linked to improvements in tissue zinc concentrations, particularly in bones and liver, which are vital for overall poultry health and productivity (El-Katcha *et al*, 2017; Kumar *et al*, 2020). Moreover, the impact of nano zinc on tissue mineral

concentrations has been reported to be dose-dependent, with higher dosages generally leading to increased mineral content (Cai *et al*, 2021; Muralisankar *et al*, 2017). Despite these promising findings, the effects of nano zinc on various aspects of poultry production are not entirely consistent. Some studies have reported no significant differences in growth performance or nutrient utilization between nano zinc and conventional zinc sources (Lina *et al*, 2009; Mao and Lien, 2017). These discrepancies may be attributed to variations in experimental

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Table 2. Zinc concentration in different dietary treatments.

Treatment	Zinc Source	Zinc added (mg/kg)	Analysed Zinc concentration # (mg/kg)
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

conditions, such as dosage, feed formulation, and environmental factors (Gopi *et al*, 2019; Liu *et al*, 2020). This study aimed to investigate the effects of biosynthesized nano zinc on growth performance, nutrient utilization, and tissue mineral concentration in Vanaraja chickens. By assessing these parameters, the research will provide valuable insights into the potential benefits and limitations of using nano zinc as a feed additive in poultry nutrition.

MATERIALS AND METHODS

The experiment was conducted at the ICAR-Directorate of Poultry Research, Hyderabad, Telangana, India, from December 2021 to February 2022. All experimental procedures complied with the institution's animal ethics committee guidelines. A total of 324-day-old Vanaraja chicks, with an average body weight of 37.35 ± 0.14 g, were randomly assigned to one of six dietary treatment groups, each comprising nine replicates. The chicks were wing-banded and housed in three-tier battery brooders. The dietary treatments included a basal diet without supplemental zinc (control, Zn_0), a basal diet supplemented with 60 mg Zn/kg from zinc oxide (Zn_60), and four diets supplemented with nano-zinc at levels of 15, 30, 45, and 60 mg Zn/kg (ZnNP_15, ZnNP_30, ZnNP_45, ZnNP_60), respectively. All diets were formulated to meet nutrient requirements based on BIS (1992) standards for poultry, except for zinc, which was supplemented as per treatment specifications. The nano-zinc powder, synthesized from *Azadirachta indica* (Neem) leaf extract using the method

outlined by Bhuyan *et al* (2015), was hand-mixed into the feed using standard procedures. Formation of nano particles were checked using particle size analysis and transmission electron microscopy (TEM) and ensured that particles were below 100nm. 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.

Chicks were provided with supplementary heat via incandescent bulbs until they reached four weeks of age. Vaccination against Newcastle disease and Infectious Bursal Disease was carried out according to the standard vaccination protocol. Daily feed intake and weekly feed residues were recorded alongside weekly body weight measurements, which were used to calculate feed consumption, weight gain, and feed conversion ratio (FCR) using standard formulas. A metabolic trial was conducted during the 6th week to assess the impact of nano-zinc on nutrient utilization. Three replicates per treatment were selected, where the daily feed intake, leftover feed, and feces excreted were measured precisely over three consecutive days. Approximately 100 g of feces from each replicate were collected daily, dried at 70°C for 20 hours, 90°C for 3 hours, and finally at 102°C for 1 hour before being weighed and stored for further analysis.

On the 56th day, one bird per replicate, with body weight closest to the mean of that replicate, was selected for slaughter, resulting in nine birds per treatment. Samples, including the right femur bone, the whole liver, and 100 g of

Table 2. Effect of feeding zinc nanoparticles on weekly feed intake, phase-wise feed intake and overall feed intake (g) of Vanaraja chicken.

Treatment	Week								Starter phase	Finisher Phase	Overall
	1	2	3	4	5	6	7	8			
Zn_0	71.56	180.30	210.28	329.33	441.31 ^b	563.80 ^b	546.93	566.56 ^b	462.14	2447.93 ^b	2910.06 ^b
Zn_60	73.77	181.45	198.87	321.39	420.65 ^{ab}	513.72 ^{ab}	566.93	514.37 ^a	454.10	2337.06 ^a	2791.15 ^a
ZnNP_15	74.89	175.89	216.98	329.28	406.83 ^a	511.41 ^{ab}	561.06	541.46 ^{ab}	467.76	2350.04 ^a	2817.80 ^{ab}
ZnNP_30	73.34	176.54	212.07	320.50	415.56 ^{ab}	520.33 ^{ab}	575.69	562.37 ^b	461.96	2394.45 ^{ab}	2856.40 ^{ab}
ZnNP_45	72.65	177.05	219.35	313.57	406.19 ^a	504.87 ^a	569.69	526.33 ^{ab}	469.05	2320.65 ^a	2789.70 ^a
ZnNP_60	72.76	178.48	221.35	321.07	411.85 ^{ab}	517.59 ^{ab}	547.74	537.30 ^{ab}	472.58	2335.56 ^a	2808.14 ^{ab}
SEM	0.68	1.69	3.35	2.68	3.19	5.66	4.11	4.55	3.85	10.62	11.66
P Value	0.829	0.932	0.445	0.555	0.011	0.034	0.239	0.002	0.808	0.002	0.016

^{a,b} Figures within a column bearing different superscripts differ significantly.

Table 3. Effect of feeding zinc nanoparticles on weekly growth, phase-wise growth and overall growth (g) of Vanaraja chicken

Treatment	Week								Starter phase	Finisher Phase	Overall
	1	2	3	4	5	6	7	8			
Zn_0	47.87	93.85	67.37	143.50	179.8	203.13	210.43	216.2	209.10	953.11 ^a	1162.21 ^a
Zn_60	47.33	94.77	68.52	153.96	193.3	204.48	235.24	249.0	210.61	1036.06 ^b	1246.67 ^b
ZnNP_15	48.65	93.80	68.91	138.56	183.3	210.85	218.95	246.7	211.35	998.35 ^{ab}	1209.71 ^{ab}
ZnNP_30	49.00	95.02	65.33	130.61	186.2	222.32	208.78	267.2	209.35	1015.15 ^{ab}	1224.50 ^{ab}
ZnNP_45	46.75	91.29	72.81	134.09	171.6	207.63	201.57	246.2	210.86	975.98 ^{ab}	1187.17 ^{ab}
ZnNP_60	48.95	98.72	65.65	129.85	178.5	222.06	214.55	243.4	213.32	988.43 ^{ab}	1201.74 ^{ab}
SEM	0.51	1.27	2.19	3.13	4.46	3.75	5.07	6.30	2.09	7.53	9.31
P Value	0.756	0.721	0.944	0.219	0.829	0.535	0.532	0.351	0.995	0.020	0.044

^{a,b} Figures within a column bearing different superscripts differ significantly.

breast muscle, were collected to analyze tissue mineral concentrations. Feed, feces, liver, and muscle samples were dried and then ashed at 600°C for 2 hours using a muffle furnace for zinc analysis. Bone samples were prepared by autoclaving, followed by overnight soaking in petroleum ether, and then dried in an oven at 102°C for 6 hours. Bone ash was obtained by heating the bone at 600°C in a muffle furnace for 4 hours. The resulting ash was dissolved in 0.5M nitric acid and diluted to 100 ml for zinc analysis, which was conducted using an Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Feed consumption

The impact of feeding nano zinc on the feed consumption of Vanaraja chickens is shown in Table 2. The average weekly feed consumption (g) for the 1–8-week period ranged from 71.56 to 74.89, 175.89 to 181.45, 198.87 to 221.35, 313.57 to 329.33, 406.19 to 441.31, 504.87 to 563.80, 546.93 to 575.69, and 526.33 to 566.56, respectively. During the starter phase, feed

consumption ranged from 461.96 to 472.58 g. In the finisher phase, it ranged from 2320.65 to 2447.93 g, and for the overall period, feed consumption varied between 2789.70 to 2910.06 g. The results demonstrated the impact of nano zinc supplementation on feed consumption in Vanaraja chickens across different growth phases. During the starter phase, feed consumption showed minor variations among different treatments, with the Zn_60 and ZnNP_60 groups showing the lowest and highest consumption, respectively. However, these differences were not statistically significant, indicating that nano zinc supplementation at various levels did not drastically alter feed intake during the early growth phase. In the finisher phase, significant differences were observed, with the Zn_0 group consuming the highest quantity of feed and the ZnNP_45 group consuming the least. This suggested that higher levels of nano zinc supplementation may lead to more efficient feed utilization during the later stages of growth, potentially due to better nutrient absorption and metabolism (Ibrahim *et al*, 2017; Asheer *et al*, 2018).

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Table 4. Effect of feeding zinc nanoparticles on weekly feed conversion ratio (FCR) phase-wise FCR and overall FCR of Vanaraja chicken

Treatment	Week								Starter phase	Finisher Phase	Overall
	1	2	3	4	5	6	7	8			
Zn_0	1.50	1.94	3.33	2.32	2.48	2.91	2.90	2.69	2.21	2.57 ^b	2.51 ^b
Zn_60	1.57	1.93	3.10	2.22	2.48	2.66	2.54	2.27	2.16	2.26 ^a	2.24 ^a
ZnNP_15	1.55	1.88	3.33	2.42	2.22	2.43	2.57	2.20	2.22	2.36 ^a	2.33 ^a
ZnNP_30	1.51	1.86	3.45	2.47	2.26	2.35	2.78	2.19	2.22	2.37 ^a	2.34 ^a
ZnNP_45	1.56	1.94	3.04	2.35	2.42	2.44	2.84	2.16	2.23	2.38 ^a	2.35 ^a
ZnNP_60	1.49	1.83	3.48	2.50	2.32	2.34	2.57	2.23	2.22	2.37 ^a	2.34 ^a
SEM	0.02	0.02	0.11	0.05	0.06	0.07	0.08	0.07	0.02	0.01	0.01
P Value	0.885	0.628	0.831	0.515	0.785	0.122	0.710	0.140	0.926	0.001	0.001

^{a,b} figures within a column bearing different superscripts differ significantly.

Table 5. Impact of Zinc Nanoparticle Supplementation on Nutrient Utilization in Vanaraja Chickens

Treatment	DM	OM	CP	EE	CF	NFE
Zn_0	66.76	69.52	57.69	64.74	10.03	77.64
Zn_60	68.85	71.52	58.26	70.45	10.50	80.02
ZnNP_15	68.88	71.64	60.78	72.22	10.13	79.27
ZnNP_30	66.73	69.59	57.51	63.68	10.30	77.80
ZnNP_45	67.48	70.20	58.32	63.17	10.08	78.40
ZnNP_60	66.72	69.53	57.70	66.97	10.00	77.58
SEM	0.56	0.52	0.87	2.04	0.08	0.50
P Value	0.777	0.749	0.929	0.792	0.587	0.717

Overall, the total feed consumption over the entire period also showed significant variations, with the Zn_0 group having the highest intake and the ZnNP_45 group the lowest. These results align with the observed trends in the finisher phase, reinforcing the idea that nano zinc can enhance feed efficiency (Mohammadi *et al*, 2015; Lee *et al*, 2021). The significant differences in feed consumption during weeks 5, 6, and 8, as well as in the finisher and overall periods, highlight the potential of nano zinc to influence feeding behaviour and efficiency. The reduced feed intake in nano zinc-supplemented groups without compromising growth performance suggests improved nutrient utilization and better

overall health (Fathi *et al*, 2018; Xia *et al*, 2019). These findings supported the hypothesis that nano zinc supplementation can optimize feed consumption patterns in poultry, particularly during the finisher phase, contributing to more efficient poultry production.

Growth

The impact of feeding zinc nanoparticles to Vanaraja chicken on growth was presented in Table 3. The respective average weekly growth (g)

ranged during the 1-8 weeks, between 46.75 to 49.00, 91.29 to 98.72, 65.33 to 72.81, 129.85 to 153.96, 171.6 to 193.3, 203.13 to 222.32, 201.57 to 235.24, and 216.2 to 267.2. The growth during starter phase ranged between 209.10 to 213.32, during finisher phase it ranged between 953.11 to 1036.06 and for the overall period, the growth ranged between 1162.21 to 1246.67. The data revealed that overall growth of negative control group was significantly ($P=0.044$) lower than the positive control group, while the nano zinc fed four groups did not differ significantly with any group. Similar trend was observed during finisher phase ($P=0.020$) with Zn positive control group significantly ($P<0.05$) higher growth compared to Zn negative control, while the nano Zn fed groups showed statistically similar growth with all other groups.

The weekly growth data indicate no significant differences among treatments across all weeks, suggesting that the presence of nano zinc did not drastically alter weekly growth patterns. This aligned with the findings of Mao and Lien (2017), who also reported no significant impact on weekly growth parameters with nano zinc supplementation. During the starter phase,

Table 6. Impact of Zinc Nanoparticle Supplementation on Zinc Bioavailability and Concentration in the Bone, Liver, and Muscle of Vanaraja Chickens

Treatment	Bio-availability (%)	Bone (ppm)	Bone ash (ppm)	Wet liver (ppm)	Dry liver (ppm)	Liver ash (ppm)	Fresh muscle (ppm)	Dry muscle (ppm)	Muscle ash (ppm)
Zn_0	49.58	99.08 ^a	224.31 ^a	32.34	114.01	465.37	9.26	36.74	906.88
Zn_60	54.72	216.87 ^{ab}	488.83 ^{ab}	35.55	123.36	563.96	10.23	38.44	987.99
ZnNP_15	50.60	240.19 ^{ab}	523.34 ^{ab}	37.30	133.66	538.51	10.77	41.28	949.35
ZnNP_30	57.89	249.86 ^b	567.47 ^b	38.92	136.48	585.51	10.48	38.59	962.23
ZnNP_45	55.21	330.15 ^b	732.50 ^b	43.15	156.13	503.84	10.40	38.60	930.54
ZnNP_60	45.19	347.28 ^b	762.04 ^b	44.27	155.59	598.64	11.18	40.62	896.49
SEM	1.82	17.86	38.60	1.33	4.80	18.64	0.77	2.99	82.45
P Value	0.396	0.01	0.01	0.081	0.060	0.303	0.991	0.999	1.000

growth performance did not significantly differ among the treatments, indicating that the initial growth phase was not markedly affected by the supplementation of nano zinc. This aligned with previous research (Ibrahim *et al*, 2017; Asheer *et al*, 2018) which found that initial growth responses to zinc supplementation were often subtle and less pronounced.

In the finisher phase, significant differences were observed, with the Zn_60 group showing the highest growth. This suggested that higher levels of zinc supplementation may lead to better growth performance during the later stages of growth, likely due to enhanced nutrient absorption and metabolism (Fathi *et al*, 2018; Xia *et al*, 2019). This is supported by the improved feed efficiency noted in the finisher phase for nano zinc-supplemented groups. Overall growth performance also showed significant differences, with the Zn_60 group outperforming others. This indicates that nano zinc supplementation can have a cumulative positive effect on growth over the entire production period. These findings align with those of Lee *et al* (2021) and Mohapatra *et al* (2019), who reported improved overall growth performance with nano zinc supplementation in poultry. The significant improvements in growth during the finisher and overall periods highlight the potential of nano zinc to enhance growth efficiency and overall productivity in poultry. Earlier investigations (Wang *et al*, 2020; El-Hack *et al*, 2020; Yousefi *et al*, 2021) also concluded that by nano zinc not only improves feed utilization but also supports better growth performance, likely through improved mineral absorption and utilization.

Feed conversion ratio

The impact of feeding zinc nanoparticles (ZnNPs) to Vanaraja chicken on feed conversion ratio (FCR) was presented in Table 4. The average weekly FCR during the 1-8 weeks ranged from 1.49 to 1.57, 1.83 to 1.97, 2.22 to 2.50, 2.22 to 2.50, 2.22 to 2.48, 2.34 to 2.91, 2.54 to 2.90, and 2.16 to 2.69. The FCR during the starter phase ranged from 2.16 to 2.23, during the finisher phase it ranged from 2.26 to 2.57, and for the overall period, it ranged from 2.24 to 2.51. The data suggested that the FCR of the zinc-negative control during the overall period was significantly ($P=0.001$) higher (or less efficient) compared to all other groups. A similar trend was observed during the finisher phase ($P=0.001$). Nano zinc-fed groups and the positive control did not differ significantly. Data related to feed consumption, growth, and FCR suggested that if feed is devoid of added zinc, the birds' growth and FCR would be negatively affected. The results of nano zinc-fed groups, which were similar to the zinc-positive control, suggest that dose reduction is possible without compromising these parameters. The data indicated that the lack of supplemental zinc adversely affects growth and feed conversion ratio (FCR), highlighting the critical importance of zinc in poultry nutrition. Ibrahim *et al* (2017) reported significant improvements in body weight gain and FCR with nano zinc supplementation in broilers. The similarity in performance between nano zinc-fed groups and the positive control suggested that nano zinc can potentially replace conventional zinc sources without compromising growth or feed efficiency (Bami *et al*, 2018). Previous studies have reported mixed results. For instance,

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Lina *et al* (2009) found no significant difference in overall growth with nano zinc supplementation, but feed consumption decreased significantly with zinc nanoparticles at 40 mg/kg. Muralisankar *et al* (2017) and Saleh *et al* (2018) reported improved growth performance and immune response with nano zinc at 40 mg/kg and 30 mg/kg, respectively.

Muralisankar *et al* (2017) and Saleh *et al* (2018) reported improved growth performance and immune response with nano zinc at 40 mg/kg and 30 mg/kg, respectively. Conversely, Zhao *et al* (2014) found significant differences in early body weights with nano zinc but no long-term benefits at higher doses. El-Katcha *et al* (2017) found that nano zinc at 30, 45, and 60 mg/kg improved growth, but at 15 mg/kg, growth was poorer than control. Ibrahim *et al* (2017) concluded that nano zinc benefits broiler diets by improving body weight gain and FCR without changing feed intake. Mohammadi *et al* (2015) found no significant differences overall due to naturally occurring zinc in the basal diet. Alkhtib *et al* (2020) observed significant early growth improvements with nano zinc but no effects from 21-35 days. Kumar *et al* (2020) found that hot melt extruded zinc significantly improved feed efficiency over 0-35 days. Cufadar *et al* (2020) and Olgun and Yildiz (2017) reported no significant differences with nano zinc in layer birds and production parameters. Mao and Lien (2017) found no significant effects on egg production with nano zinc in laying birds.

Nutrient utilization

The impact of zinc nanoparticle supplementation on nutrient utilization in Vanaraja chickens is displayed in Table 5. Feeding zinc nanoparticles had no significant effect on the utilization of dietary components, including dry matter, organic matter, crude protein, crude fiber, ether extract, and nitrogen-free extract, as shown in Table 4. Digestibility of dry matter and organic matter remained similar across the treatment groups, with no significant differences observed ($P>0.05$). The ZnNP_15 group had the highest values for digestibility of both DM (68.88%) and OM (71.64%), slightly higher than the Zn_60 group. The improved digestibility in the ZnNP_15 group aligns with findings by Ibrahim *et al* (2017),

who reported enhanced nutrient absorption with nano zinc supplementation.

The study found that crude protein (CP) and ether extract (EE) digestibility were highest in the ZnNP_15 group, with CP digestibility reaching 60.78% and EE at 72.22%, although the differences were not statistically significant ($P>0.05$). These results suggest that nano zinc, especially at lower doses, may enhance protein and lipid utilization. Findings are consistent with Asheer *et al* (2018) and Saleh *et al* (2018), who reported benefits in protein efficiency and lipid metabolism with nano zinc supplementation. Similarly, nitrogen-free extract (NFE) digestibility was highest in the Zn_60 and ZnNP_15 groups, supporting carbohydrate metabolism. Although crude fiber (CF) digestibility and other results were unaffected, the improved protein and fat digestibility with nano zinc, particularly at 15 ppm, highlights its potential to enhance nutrient utilization in Vanaraja chickens. This aligns with prior research, such as Ibrahim *et al* (2017) and Gopi *et al* (2019), who observed similar improvements in nutrient retention and utilization with nano zinc.

Bioavailability and Tissue content of zinc

The impact of feeding zinc nanoparticles (ZnNPs) to Vanaraja chickens on zinc bioavailability and tissue concentrations is detailed in Table 6. Although bioavailability differences were not statistically significant ($P>0.05$), the ZnNP_30 group recorded the highest bioavailability at 57.89%, aligning with Ibrahim *et al* (2017), who also observed enhanced bioavailability with nano zinc. Significant increases in bone and bone ash zinc content were found, with the highest values in the ZnNP_60 group (347.28 ppm in bone and 762.04 ppm in bone ash), suggesting a notable impact of ZnNPs on zinc deposition in skeletal tissues. These findings are consistent with Asheer *et al* (2018), who reported similar results in broilers. Zinc levels in liver tissues, while not statistically significant, were also highest in the ZnNP_60 group, indicating enhanced zinc retention. This aligns with El-Katcha *et al* (2017), who found increased tissue zinc levels with nano zinc. Muscle tissue showed no significant differences,

echoing the findings of Mohammadi *et al* (2015). Overall, higher ZnNP doses significantly improved zinc content in bone, with potential benefits in liver zinc retention.

CONCLUSION

The results in terms of growth and FCR indicated that, feeding of nanoparticles at lower doses (25, 50, and 75%) was as beneficial as that of feeding inorganic zinc at full dose. The positive control group and all the four nano zinc fed groups have shown significantly ($P < 0.05$) better performance in terms of growth and FCR. Nanoparticles of zinc at lower levels compared to inorganic zinc, were found to be beneficial in reducing the dietary doses. These findings supported the use of ZnNPs as a viable alternative to conventional zinc sources in poultry diets, potentially allowing for reduced zinc supplementation levels without compromising tissue zinc levels.

ETHICAL STATEMENT

The animal experiment followed the guidelines set by the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) for the use of animals in scientific research in India. The experimental protocol was reviewed and approved by the Institute's Animal Ethics Committee, with the approval number IAEC/DPR/21/01.

ACKNOWLEDGEMENT

The authors express their gratitude to the Director of the ICAR-Directorate of Poultry Research, Hyderabad, for providing the necessary farm, laboratory, and logistical support for conducting this study.

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Received on 22/8/2024 Accepted on 19/10/2024