Indian J Anim Health (2025), 64(2)- Special Issue: 442 - 450
DOI: https://doi.org/10.36062/ijah.2025.spl.01625

Research Article

Effect of green-synthesised zinc oxide nanoparticles supplementation on laying performance and egg quality traits of Japanese quails

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Abstract

The escalating cost of poultry production and market competition necessitate alternative strategies to enhance productivity and profitability. Japanese quail (*Coturnix coturnix japonica*) are increasingly reared for egg and meat production due to their early sexual maturity, high egg output, and resilience to environmental stress. Zinc, a crucial trace element involved in enzymatic functions, protein synthesis, and reproductive processes, is often inadequately bioavailable in conventional dietary sources. Nanotechnology offers a potential solution; zinc oxide nanoparticles (ZnO-NPs) exhibit higher bioavailability, and green synthesis using *Moringa oleifera* leaf extracts provides a cost-effective and sustainable approach. This study evaluated the effects of graded dietary supplementation of ZnO-NPs (0–50 mg/kg) on egg production and quality in 240 laying Japanese quails over 60 days. Birds were randomly allocated into six treatment groups (T1-T6). T1 (control) offered basal ration only, whereas, T2 (10 mg/kg ZnO-NPs), T3 (20 mg/kg ZnO-NPs), T4 (30 mg/kg ZnO-NPs), T5 (40 mg/kg ZnO-NPs), and T6 (50 mg/kg ZnO-NPs).The parameters including egg production, egg weight, yolk height, yolk colour, eggshell weight, thickness, and internal quality indices were recorded. Supplementation of ZnO-NPs at 30–50 mg/kg significantly enhanced total egg production, egg weight, yolk height, yolk colour, and eggshell characteristics ($P<0.05$), while albumen quality, Haugh unit, and yolk/albumen indices remained unaffected. The findings suggest that green-synthesized ZnO-NPs are an effective dietary additive to improve reproductive performance and external egg quality in Japanese quails, offering a promising strategy for cost-effective and sustainable poultry production.

Keywords: Egg quality, Egg production, Japanese quail, *Moringa oleifera*, Zinc oxide nanoparticles

Highlights

- ZnO-NPs (30–50 mg/kg) in diet enhanced egg production and egg weight in Japanese quails
- Supplementation improved yolk height, color, shell weight, and thickness in quail eggs
- Moringa-based ZnO-NPs showed high bioavailability, boosting enzymes, minerals, productivity

INTRODUCTION

With rising production costs and intensifying competition in broiler and layer sectors, poultry farmers are exploring alternative ventures to sustain profitability. Quail farming has emerged as a viable option for diversification, offering higher returns with relatively low input requirements. Japanese quail (*Coturnix coturnix japonica*), belonging to the family Phasianidae and commonly referred to as "Bater," are extensively reared for commercial meat and egg production. Introduced in India in 1974 by the Central Avian Research Institute (CARI), Bareilly, these birds are hardy, easy to handle, and adaptable to diverse agro-climatic conditions (Singh & Johari, 1977). They exhibit rapid sexual maturation, short generation intervals, high reproductive capacity, and superior egg production compared to other poultry species (Narinc et al., 2014; Molino et al., 2015). Moreover, quails possess robust immune systems and are more resilient to environmental stress (Nasaka et al., 2017).

Zinc is an essential trace element critical for growth, metabolism, reproduction, immunity, and enzymatic regulation, functioning as a cofactor in over 200 Zn-metalloenzymes (Liu et al., 2018; De Grande et al., 2021). Japanese quails are particularly susceptible to zinc deficiency, which can impair feathering, egg production, immunity, and maturation (Sahin et al., 2005; Mohamed et al., 2019). Although organic zinc offers superior bioavailability compared to inorganic sources (Feng et al., 2009; Dong et al., 2023).

Nanotechnology offers a promising solution, with zinc oxide nanoparticles (ZnO-NPs) demonstrating higher bioavailability due to their small size, large surface area, and unique physicochemical properties (Jeevanandam et al., 2018; Patra & Lalhriatpuii, 2020). Green synthesis using *Moringa oleifera* leaf extracts,

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rich in sterols, flavonoids, alkaloids, saponins, and terpenoids, provides a cost-effective and eco-friendly approach, with bioactive compounds serving as natural reducing, capping and stabilizing agents during nanoparticle formation (Abbas et al., 2018; Islam et al., 2021). Abbasi et al. (2022) found that supplementation with nano-ZnO improved eggshell thickness, egg weight, hatchability/fertility and maximum egg-production achieved at ~67 mg/kg Zn from nano-ZnO in quail birds. *Moringa oleifera* leaf powder extract-based zinc nanoparticles (ZnNPs-MLPE) worked as an effective natural growth enhancer for producing healthier poultry products (El-Abbasy et al., 2025). Despite this, research on the application of ZnO-NPs in quail nutrition is scarce. Therefore, the present study was undertaken to evaluate the effect of feeding green-synthesized ZnO-NPs on the laying performance and egg quality traits of Japanese quails.

MATERIALS AND METHODS

Preparation of zinc oxide nanoparticles: Leaf extracts of *Moringa oleifera* were employed for the green synthesis of Zn oxide nanoparticles (ZnO-NPs). The leaves of *Moringa oleifera* are rich in diverse phytochemicals, including sterols, tannins, flavonoids, alkaloids, saponins, and terpenoids. These bioactive compounds function as natural reducing and stabilizing agents, thereby facilitating the synthesis process and contributing to the reduction in particle size of the nanoparticles. In the present study, ZnO-NPs were synthesized in the laboratory using *Moringa oleifera* leaf extract following the methodology described by Kanti (2016). First of all, 50 mL of *Moringa oleifera* leaves extract was taken in a 250 mL beaker and heated to 60-80! on hot plate. Then 5 g of zinc acetate was added to the solution. This whole mixture was then boiled using hot plate magnetic stirrer till it reduced to light yellow color paste. Further, the paste obtained was collected in ceramic crucible and heated in muffle furnace at 400ºC for 2 hrs. The white color powder was obtained using the mortar-pestle.

The particle size and size distribution of the obtained ZnO-NPs were investigated using High-resolution transmission electron microscopy (HRTEM) and Transmission electron microscopy (TEM) using JEOL-JEM-F20 machine at the Department of Physics, Research and Development Unit, Indian Institute of Technology (IIT), Patna, India. HRTEM and TEM images of ZnO-NPs indicated the hexagonal shape of the obtained nano crystals of ZnO and a particle size of 70 nm. Further, the synthesized nanoparticles were subsequently utilized as dietary supplements as an additive for quails.

Feeding, management and dietary treatment: The experiment was carried out for a period of 60 days on laying Japanese quails at the Poultry Research and Training Centre, Bihar Animal Sciences University, Patna, India. A total of 240 quails were randomly distributed into six dietary treatment groups, each consisting of 40 birds with five replicates of eight quails per replicate. The experimental design included iso-nutritive diets as follows: T1 (control), T2 (10 mg/kg ZnO-NPs), T3 (20 mg/kg ZnO-NPs), T4 (30 mg/kg ZnO-NPs), T5 (40 mg/kg ZnO-NPs), and T6 (50 mg/kg ZnO-NPs). The birds were housed in a cage system under adequate lighting and ventilation conditions throughout the trial. Standard managemental practices, including the prescribed vaccination schedule, were strictly followed. During the entire experimental period, birds received daily allowance of feed along with ad libitum access to clean drinking water as per their requirements.

Performance parameters and egg quality characteristics: Egg production and egg quality parameters were systematically evaluated during the experimental period. Eggs were collected twice daily (morning and evening) and the total number of eggs produced per replicate was divided by the number of quails in that replicate to determine egg production rate. Egg weight was recorded throughout the study to assess both production performance and egg quality traits. The average number of eggs for each dietary treatment was calculated from 50 to 110 days of age.

For egg quality assessment, two egg per replicate were sampled on a fortnightly basis, resulting in a total of 40 eggs per group for evaluation of external and internal quality traits. Eggs were cleaned using cotton or tissue paper and weighed individually prior to analysis. The parameters studied included whole egg weight (g), albumen height (mm), yolk width (cm), yolk height (mm), yolk colour, yolk weight (g), eggshell weight (g), eggshell thickness (mm), yolk percentage, eggshell percentage, shape index (%), yolk index (%), albumen index (%), and Haugh unit (HU). The HU index was calculated based on the height (H) of the thick albumen (mm) and the weight (W) of the whole egg (g), using the formula: $HU=100 \log (H-1.7 W^{0.37}+7.6)$, as described by Haugh (1937).

Laboratory analysis: The feed ingredients, compounded diets, and feed residues were analyzed

Table 1. Chemical composition of feed ingredients used in experiment (% on DM basis)

Ingredients	DM	OM	CP	EE	CF	TA	AIA	NFE	Ca	P
Yellow maize	91.18	97.11	9.79	4.19	2.26	2.89	1.16	80.87	0.11	0.43
Soyabean meal	91.66	94.02	46.04	0.46	5.61	5.98	1.19	41.91	0.36	0.64

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fibre; TA, total ash; AIA, acid insoluble ash; NFE, nitrogen free extract; Ca, calcium; P, phosphorus.

Table 2. Percentage composition of experimental diets

Ingredients	Layer feed
Yellow maize	33.00
Soya bean meal	24.00
Wheat bran	20.00
De-oiled rice bran	9.00
Vegetable oil	3.00
Common salt	0.40
Di-calcium Phosphate	1.00
Calcite powder	8.00
DL-Methionine	0.20
Lysine	0.40
Mineral mixture*	0.50
Premix*	0.50
Total	100
Calculated value	
CP (%)	18.10
ME (kcal/kg)	2565.00
CF (%)	8.36
Ca (%)	3.15
Av. P (%)	0.64
Lysine (%)	0.73
Methionine (%)	0.34

CP, crude protein; ME, metabolizable energy; CF, crude fibre; Ca, calcium; Av. P, available phosphorus.

*Composition of mineral mixture and premix: Vitamin A (7,00,000 I.U.), Vitamin D_3 (70,000 I.U.), Vitamin E (250 mg), Nicotinamide (1000 mg), Vitamin B_1 (2 mg), Vitamin B_2 (4 mg), Niacin (60 mg), Pantothenic acid (10 mg), Cyanocobalamin (10 µg), Choline (500 mg), Cobalt (150 mg), Copper (1200 mg), Iodine (325 mg), Iron (1500 mg), Potassium (100 mg), Magnesium (6000 mg), Manganese (1500 mg), Selenium (10 mg), Sodium (5.9 mg), Sulfur (0.72 %), Zinc (9600 mg), Calcium (25.5%) and Phosphorus (12.75%).

for their nutrient composition. Parameters included dry matter (DM; ID 930.15), organic matter (OM), total ash and acid-insoluble ash (ID 942.05), crude protein (CP; N × 6.25, ID 954.01), ether extract (EE; ID 920.39), crude fibre, and nitrogen-free extract, following the standard procedures of the Association of Official Analytical Chemists [AOAC] (1995). Calcium and phosphorus concentrations were estimated using the modified method of Talapatra et al. (1940). Standard basal diets for Japanese quails were formulated by blending the analyzed feed ingredients to meet nutrient requirements in accordance with the Indian Council of Agricultural Research [ICAR] (2013) recommendations. Experimental diets were prepared on the basis of crude protein values and standard metabolizable energy requirements. To ensure accuracy, all formulated rations were subsequently reanalyzed in the laboratory for their proximate composition using the same AOAC protocols. Zinc levels in the experimental diets were analyzed at the Animal Feed Analytical and Quality Assurance Laboratory, Namakkal, TANUVAS, Tamil Nadu. The measured concentrations were 99.4, 104.0, 108.7, 113.1, 117.7, and 122.3 mg/kg for T1, T2, T3, T4, T5, and T6 diets, respectively.

Statistical analysis

Statistical analyses were carried out using Statistical Package for the Social Sciences [SPSS] software (version 20.0) (2011). Data were subjected to analysis of variance (ANOVA) under a generalized linear model to compare the effects among different treatment groups. Significant differences between the control and treatment means were further evaluated using Duncan's multiple range test. All statistical procedures were performed following the methodology outlined by Snedecor & Cochran (1994).

RESULTS

The chemical composition of the feed ingredients used for basal diet preparation and compounded feed offered to the quail birds is presented in Table 1. The percentage composition of experimental diet is depicted in Table 2.

Egg production performance: During the first fortnight, egg production ranged from 38.75 to 44.75 eggs across treatments, with no significant differences (P=0.204). In the subsequent fortnights, birds receiving higher levels of ZnO-NPs (T4–T6) generally produced more eggs. Significant differences were observed in the third (P=0.016) and fourth (P=0.003) fortnights, with T4 showing the highest production, though differences were marginally significant (P=0.077)

Table 3. Effect of supplementing ZnO-NPs on egg production in Japanese quail

Fortnight	T_1	T_2	T_3	T_4	T_5	T_6	SEM	P-value
1st	38.75	44.75	43.75	42.00	43.25	44.75	2.515	0.204
2nd	93.00 ^{a}	90.75 ^{a}	93.25 ^{a}	96.75 ^{ab}	95.00 ^{ab}	101.50 ^{b}	3.421	0.077
3rd	92.75 ^{a}	98.50 ^{ab}	101.50 ^{abc}	109.00 ^{c}	102.75 ^{bc}	106.25 ^{bc}	4.191	0.016
4th	94.50 ^{a}	98.75 ^{ab}	103.25 ^{bc}	111.50 ^{c}	107.00 ^{bc}	106.25 ^{bc}	3.713	0.003
1st – 4th fortnight total egg production (nos.)	319.00 ^{a}	332.75 ^{ab}	341.75 ^{bc}	359.25 ^{c}	348.00 ^{bc}	358.75 ^{c}	8.871	0.002

(Table 3). Cumulatively, total egg production over the four fortnights was significantly higher in T4 (359.25 eggs) and T6 (358.75 eggs) compared to the control (T1, 319 eggs), indicating that dietary supplementation of ZnO-NPs improved laying performance in quails.

The total input cost per bird was calculated based on total experimental feed cost and egg production. Treatment group (T4) decreased the production cost by enhancement of total egg production. However, the feed cost rupees per egg were minimum in T4 group followed by T6, T5, T3, T2, and T1 group. Hence, T4 group was found to be most economical among all the treatment groups.

Egg quality traits: Egg weight showed an increasing trend with ZnO-NPs supplementation (Table 4). During the first three fortnights, differences among treatments were not significant (P>0.05). In the fourth fortnight, T3 recorded the highest egg weight (11.734 g), which was significantly higher than T2, T4, and T5 (P=0.056). The cumulative egg weight over the four fortnights was significantly higher in T3 (10.644 g) and T6 (10.496 g) compared to the control (T1, 10.288 g; P=0.018). This indicates that intermediate and higher levels of ZnO-NPs positively influenced egg mass.Albumen height varied slightly across treatments but was not significantly affected by ZnO-NPs supplementation in any fortnight (P>0.05). The overall mean ranged from 4.24 mm (T4) to 4.77 mm (T3), suggesting that albumen quality remained stable across different levels of supplementation. No significant differences were observed in yolk width (cm) among treatments during the experiment (P>0.05), with values ranging from 2.32 to 2.37 cm. Moreover, T4 exhibited significantly higher yolk height (mm) in the second fortnight (12.48 mm) compared to the control and lower supplementation levels (P<0.001). The cumulative yolk height was also significantly higher in T4 (11.622 mm) and T6 (11.544 mm; P=0.002), indicating improved yolk development.Yolk colour was significantly enhanced in T4 during the fourth fortnight (5.0) compared to T1 (4.2; P=0.043). The overall mean yolk colour over four fortnights was significantly higher in T4 (5.0) than in T1 (4.6; P=0.031), suggesting that ZnO-NPs may influence yolk pigmentation. Yolk weight showed slight variations, with T6 recording the highest value in the first fortnight (3.17 g; P = 0.015). However, cumulative yolk weight across four fortnights did not differ significantly among groups (P>0.05). Egg shell weight (g) in T6 showed significantly higher shell weight in the third fortnight (1.248 g; P=0.026). The egg shell thickness (mm) in T4 and T5 had significantly higher shell thickness in the second fortnight (0.282–0.284 mm; P=0.036).

However, egg shell percentage in T5 and T6 showed improved shell percentage in the third and fourth fortnights (P<0.05). These results indicate that ZnO-NPs supplementation enhances shell quality, particularly at higher doses.Haugh unit values were not significantly influenced by ZnO-NPs supplementation (P>0.05), ranging from 89.21 to 91.53 across treatments, indicating stable albumen quality. No significant differences were observed in the egg shape index among treatments (P>0.05), suggesting that ZnO-NPs did not affect egg shape. The yolk index in T4 and T5 showed higher yolk index values in the second fortnight (54.772% and 54.146%, respectively; P=0.026). Overall, differences among treatments were not statistically significant (P>0.05). The albumen index showed no significant differences across treatments for albumen index (P>0.05), indicating that albumen quality remained unaffected.

Dietary supplementation of ZnO-NPs significantly improved egg weight, yolk height, yolk colour, and certain shell characteristics, particularly at 30–50 mg/kg, while other internal traits such as albumen height, Haugh unit, and yolk/albumen indices remained largely unaffected. These results suggest a beneficial role of

Table 4. Effect of supplementing ZnO-NPs on egg quality traits at fortnightly intervals in Japanese quail

Fortnight	T_1	T_2	T_3	T_4	T_5	T_6	SEM	P-value
Egg weight (g)								
1st	9.77	9.06	9.36	9.39	9.04	9.99	0.408	0.166
2nd	10.26	10.01	9.79	10.21	10.61	10.27	0.559	0.782
3rd	10.37	10.97	11.69	10.66	10.18	10.98	0.610	0.214
4th	10.74 ^{ab}	9.814 ^{a}	11.734 ^{b}	10.156 ^{a}	10.082 ^{a}	10.732 ^{ab}	0.613	0.056
1st – 4th fortnight	10.288 ^{abc}	9.966 ^{a}	10.644 ^{c}	10.104 ^{ab}	9.982 ^{a}	10.496 ^{bc}	0.213	0.018
Albumen height (mm)								
1st	4.22	3.78	4.38	3.72	4.10	4.14	0.436	0.633
2nd	5.14	5.26	5.38	4.98	5.34	4.54	0.454	0.466
3rd	4.57	4.42	4.94	4.08	4.34	4.47	0.375	0.364
4th	4.50	4.21	4.37	4.16	3.99	4.55	0.563	0.913
1st – 4th fortnight	4.61	4.42	4.77	4.24	4.44	4.43	0.243	0.372
Yolk width (cm)								
1st	2.40	2.30	2.32	2.38	2.34	2.42	0.108	0.853
2nd	2.16	2.24	2.22	2.28	2.32	2.30	0.074	0.310
3rd	2.30	2.48	2.44	2.30	2.34	2.40	0.098	0.344
4th	2.40	2.32	2.48	2.31	2.27	2.29	0.098	0.290
1st – 4th fortnight	2.32	2.34	2.37	2.32	2.32	2.36	0.046	0.817
Yolk height (mm)								
1st	9.72	9.26	9.84	9.82	8.80	10.06	0.504	0.170
2nd	10.73 ^{a}	10.42 ^{ab}	11.48 ^{bc}	12.48 ^{cd}	12.54 ^{d}	12.31 ^{cd}	0.464	<0.001
3rd	11.97	11.64	12.16	12.23	11.65	12.30	0.371	0.326
4th	11.53	11.28	11.86	11.96	12.13	11.50	0.397	0.288
1st – 4th fortnight	10.986 ^{ab}	10.652 a	11.336 ^{bc}	11.622 ^{c}	11.28 ^{bc}	11.544 ^{c}	0.227	0.002
Yolk colour								
1st	5.00	5.00	5.00	5.00	5.00	4.80	0.216	0.916
2nd	4.60	4.60	4.80	5.20	5.00	4.40	0.346	0.245
3rd	4.60	5.00	4.60	5.00	4.80	4.60	0.374	0.735
4th	4.2 ^{a}	5.2 ^{b}	5.00 ^{b}	4.8 ^{ab}	4.8 ^{ab}	5.00 ^{b}	0.294	0.043
1st – 4th fortnight	4.6 ^{a}	4.95 ^{bc}	4.85 ^{abc}	5.0 ^{c}	4.9 ^{bc}	4.7 ^{ab}	0.126	0.031
Yolk weight (g)								
1st	2.842 ^{a}	2.736 ^{a}	2.6 ^{a}	2.818 ^{a}	2.69 ^{a}	3.17 ^{b}	0.148	0.015
2nd	3.08	2.99	2.99	3.28	3.29	3.04	0.216	0.554
3rd	3.19	3.16	3.62	3.30	3.24	3.41	0.206	0.278
4th	3.20	3.00	3.19	2.97	2.86	2.99	0.293	0.833
1st – 4th fortnight	3.08	2.97	3.10	3.09	3.02	3.15	0.095	0.513
Egg shell weight (g)								
1st	1.01	1.03	1.00	0.98	1.05	1.06	0.058	0.731
2nd	1.24	1.20	1.15	1.10	1.15	1.08	0.075	0.296
3rd	0.954 ^{a}	1.004 ^{ab}	1.166 ^{bc}	1.056 ^{abc}	1.066 ^{abc}	1.248 ^{c}	0.086	0.026
4th	1.07	1.10	1.19	1.19	1.21	1.21	0.080	0.345
1st – 4th fortnight	1.07	1.09	1.13	1.08	1.12	1.15	0.037	0.268

Cont. Table 4.

Table 4., Cont. ...

Fortnight	T_1	T_2	T_3	T_4	T_5	T_6	SEM	P-value
Egg shell thickness (mm)								
1st	0.24	0.23	0.21	0.19	0.21	0.22	0.019	0.125
2nd	0.218 ^{a}	0.242 ^{ab}	0.244 ^{ab}	0.282 ^{b}	0.284 ^{b}	0.262 ^{ab}	0.021	0.036
3rd	0.246 ^{c}	0.228 ^{abc}	0.242 ^{bc}	0.212 ^{a}	0.216 ^{ab}	0.236 ^{abc}	0.013	0.076
4th	0.19	0.23	0.22	0.37	0.23	0.22	0.090	0.424
1st – 4th fortnight	0.22	0.24	0.23	0.27	0.24	0.23	0.025	0.638
Haug unit								
1st	89.548	87.684	90.498	86.888	89.438	88.928	2.389	0.690
2nd	94.022	94.78	95.512	93.158	94.666	90.604	2.260	0.339
3rd	91.002	89.712	91.852	87.866	89.962	89.976	1.896	0.435
4th	90.004	89.37	88.238	88.916	87.984	90.566	3.146	0.958
1st – 4th fortnight	91.15	90.38	91.53	89.21	90.51	90.01	1.278	0.542
Egg yolk (%)								
1st	29.02	30.78	27.82	30.02	29.74	31.76	1.964	0.455
2nd	30.10	29.89	30.64	32.12	30.83	29.59	1.139	0.309
3rd	30.73	28.85	30.96	31.09	31.75	31.20	1.345	0.386
4th	29.76	30.62	27.28	29.38	28.40	27.85	2.261	0.688
1st – 4th fortnight	29.90	30.03	29.18	30.65	30.17	30.10	0.940	0.757
Egg shell weight (%)								
1st	10.34	11.48	10.66	10.49	11.56	10.64	0.538	0.131
2nd	12.14	12.01	11.78	10.82	10.82	10.60	0.722	0.147
3rd	9.228 ^{a}	9.18 ^{a}	9.984 ^{a}	9.866 ^{a}	10.442 ^{ab}	11.384 ^{b}	0.576	0.008
4th	9.98 ^{a}	11.15 ^{ab}	10.144 ^{a}	11.666 ^{b}	11.964 ^{b}	11.438 ^{ab}	0.677	0.034
1st – 4th fortnight	10.42	10.96	10.64	10.71	11.19	11.02	0.402	0.440
Shape index (%)								
1st	77.52	76.16	75.22	79.73	79.35	77.19	1.841	0.146
2nd	80.54	69.00	79.83	80.12	81.89	83.01	5.836	0.223
3rd	78.42	80.82	80.08	78.28	78.35	78.75	3.211	0.949
4th	87.94	78.62	85.10	78.56	85.38	80.99	4.224	0.166
1st – 4th fortnight	81.10	76.15	80.06	79.17	81.24	79.98	2.033	0.176
Yolk index (%)								
1st	41.60	40.30	42.46	41.26	37.80	41.63	3.156	0.742
2nd	49.84 ^{ab}	46.624 ^{a}	51.824 ^{ab}	54.772 ^{b}	54.146 ^{b}	53.57 ^{b}	2.486	0.026
3rd	52.05	47.58	49.93	53.18	49.83	51.35	2.385	0.272
4th	48.08	48.74	48.14	51.91	53.64	50.27	2.666	0.246
1st – 4th fortnight	47.89	45.81	48.09	50.28	48.85	49.20	1.450	0.091
Albumin index (%)								
1st	11.57	10.98	12.21	10.79	11.99	11.68	1.635	0.945
2nd	16.01	17.27	16.53	16.00	16.11	14.03	1.774	0.602
3rd	13.69	12.56	13.51	12.11	13.13	13.76	1.306	0.756
4th	12.88	12.77	11.70	12.04	12.33	13.62	1.838	0.921
1st – 4th fortnight	13.54	13.40	13.48	12.73	13.39	13.27	0.882	0.950

^{abc}Values with different superscripts in a row differ significantly ($P<0.05$; $P<0.01$)

ZnO-NPs in enhancing specific aspects of egg quality in Japanese quails.

DISCUSSIONS

The enhanced egg production may be attributed to the essential role of zinc in several physiological and reproductive processes. Zn acts as a cofactor for over 200 metalloenzymes involved in protein and nucleic acid metabolism, hormone regulation, and antioxidant defense, all of which are critical for follicular development and ovulation in birds (Liu et al., 2013). Moreover, the nano form of Zn offers higher bioavailability due to its smaller particle size and greater surface area, facilitating efficient absorption and utilization in metabolic pathways (Patra & Lalhriatpuii, 2020). Zn-NPs supplementation at 0.2 g/kg diet had a positive effect on the performance and physiological status of growing Japanese quails (Reda et al., 2021).

These findings are consistent with previous reports indicating that dietary Zn supplementation improves egg production in poultry. Sahin et al. (2005) observed increased egg output in Japanese quails supplemented with Zn, and Mohamed et al. (2019) reported enhanced reproductive performance with nano Zn supplementation. The results of the present study suggest that ZnO-NPs at 30–50 mg/kg can be an effective feed additive to enhance egg production in Japanese quails, likely through improved nutrient utilization, enzyme activation, and reproductive efficiency and proved to be economical and cost-effective compared to inorganic zinc sources.

The supplementation of ZnO-NPs influenced several egg quality traits in Japanese quails, though the effects varied among parameters. The improved egg weight may be attributed to zinc's role in protein synthesis and enzyme activation, which enhance yolk and albumen formation (Sahin et al., 2005; Liu et al., 2013). There were no significant differences in albumen height and Haugh unit across treatments (P>0.05), indicating that internal egg quality remained stable. Previous studies have also reported minimal effect of ZnO-NPs on albumen quality, suggesting that moderate zinc supplementation primarily affects growth and reproductive output rather than internal egg viscosity (Mohamed et al., 2019). Yolk height and yolk colour were significantly improved in T4 and T5 during specific fortnights (P<0.05). Yolk height was increased in T4 (12.48 mm) in the second fortnight, while yolk colour intensity was higher in T4 in the fourth fortnight. These improvements may be linked to zinc's involvement in lipid metabolism and carotenoid deposition in the yolk (Patra & Lalhriatpuii, 2020). Yolk weight, however, did not differ significantly across treatments over the entire period.

Egg shell characteristics includes egg shell weight and thickness were enhanced in T3–T6, particularly in the third fortnight (P<0.05). ZnO-NPs likely improved shell formation through its role in calcium metabolism and activation of carbonic anhydrase, essential for shell mineralization (Sahin et al., 2005; De Grande et al., 2021). Shell percentage also increased in T5 and T6, supporting better structural integrity. The shape index, yolk index, and albumen indexparameters showed minor variations with no significant differences (P>0.05), indicating that ZnO-NPs did not adversely affect egg shape or proportional yolk and albumen quality.

The study indicates that ZnO-NPs supplementation at 20–50 mg/kg can improve external egg quality traits, especially egg weight, yolk height, yolk colour, and shell quality, while maintaining internal egg quality. These results align with earlier findings on the benefits of nano zinc in poultry diets for enhancing productivity and egg quality (Sahin et al., 2005; Mohamed et al., 2019; Patra & Lalhriatpuii, 2020).

Dietary supplementation of zinc oxide nanoparticles (ZnO-NPs) at 30 mg/kg feed significantly improved the productive performance of Japanese quails. Birds receiving ZnO-NPs exhibited higher egg production, increased egg weight, enhanced yolk height and colour, and improved eggshell quality, while internal egg quality traits, such as albumen height, Haugh unit, and yolk index, were unaffected. The observed benefits are attributed to the greater bioavailability of nano zinc, supporting enzymatic functions, protein synthesis, and mineral metabolism. ZnO-NPs (@ 30 mg/kg) thus represent an effective strategy for enhancing quail productivity and egg quality, which can be adopted for economic production in quail farming practices.

Conflicts of interest: The authors declare that there are no conflicts of interest relevant to the content of this paper.

Author's contributions: KK: Conceived and designed research; SKi: Conducted experiments; SKr: Contributed reagents and sample analysis; AR: Analysed experimental data; PKS: Writing the manuscript; RRKS: Critically revising the manuscript. All authors read and approved the manuscript.

Data availability statement: Data of research will be made available with the corresponding author on demand.

Ethical statement: The procedures in this study were approved vide No. IAEC/BVC/2024/24 dated- 22/07/2024 (1365/GO/Re/S/L/10/CPCSEA)by the Institutional Animal Ethical Committee, Bihar Veterinary College, Patna, Bihar, India.

Use of artificial intelligence tools: During the preparation of this work, the author(s) used ChatGPT AI tools for restructuring and concising the sentences to some extent. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to the Vice Chancellor of Bihar Animal Sciences University, Patna, India, for providing the essential facilities and financial support to carry out this experiment.

REFERENCES

Abbas, R. K., Elsharbasy, F. S., & Fadlelmula, A. A. (2018). Nutritional values of *Moringa oleifera*, total protein, amino acid, vitamins, minerals, carbohydrates, total fat and crude fiber, under the semi-arid conditions of Sudan. *Journal of Microbial & Biochemical Technology*, *10*(2). https://doi.org/10.4172/1948-5948.1000396

Abbasi, M., Dastar, B., Afzali, N., Shargh, M. S., & Hashemi, S. R. (2022). The effects of nano and micro particle size of zinc oxide on performance, fertility, hatchability, and egg quality characteristics in laying Japanese quail. *Biological Trace Element Research, 200*(5), 2338-2348. https://doi.org/10.1007/s12011-021-02848-2

Association of Official Analytical Chemists. (1995). *Methods of Analysis* (16th edn, Vol. 1).

De Grande, A., Ducatelle, R., Delezie, E., Rapp, C., De Smet, S., Michiels, J., Haesebrouck, F., Van Immerseel, F., & Leleu, S. (2021). Effect of vitamin E level and dietary zinc source on performance and intestinal health parameters in male broilers exposed to a temperature challenge in the finisher period. *Journal of Animal Physiology and Animal Nutrition*, *105*(4), 777-786. https://doi.org/10.1111/jpn.13492

Dong, W., Fan, Z., Li, P., Liu, J., Sun, G., Peng, N., Liang, Y., & Zhao, S. (2023). Optimizing the scale-up production of fermented astragalus and its benefits to the performance and egg quality of laying hens. *Frontiers in Microbiology*, *14*, 1165644. https://doi.org/10.3389/fmicb.2023.1165644

El-Abbasy, M. M., Aldhalmi, A. K., Ashour, E. A., Bassiony, S. S., Kamal, M., Alqhtani, A. H., Abou-Kassem, D. E., Elolimy, A. A., El-Hack, M. E. A., & Swelum, A. A. (2025). Enhancing broiler growth and carcass quality: impact of diets enriched with *Moringa oleifera* leaf powder conjugated with zinc nanoparticles. *Poultry Science, 104*(1), 104519. https://doi.org/10.1016/j.psj.2024.104519

Feng, J., Q, W., MA, Niu, H. H., Wu, X. M., Wang, Y., & Feng, J. (2009). Effects of zinc glycine chelate on growth, hematological, and immunological characteristics in broilers. *Biological Trace Element Research*, *133*(2), 2 03–211. https://doi.org/10.1007/s12011-009-8431-9

Haugh, H. (1937). The Haugh unit for measuring egg quality. *The United States Egg and Poultry Magazine, 43*, 552-555.

Indian Council of Agricultural Research. (2013). *Nutrient requirements of poultry*. http://krishi.icar.gov.in/jspui/handle/123456789/9857.

Islam, Z., Islam, S. M. R., Hossen, F., Mahtab-Ul-Islam, K., Hasan, M. R., & Karim, R. (2021). *Moringa oleifera* is a prominent source of nutrients with potential health benefits. *International Journal of Food Science*, *2021*, 6627265. https://doi.org/10.1155/2021/6627265

Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein Journal of Nanotechnology*, *9*, 1050–1074. https://doi.org/10.3762/bjnano.9.98

Kanti, R. (2016). *Effect of supplementation of zinc nano particles on growth and health status of Wistar rats (Rattus norvegicus).* (Master's thesis). IVRI, Izatnagar.

Liu, S., Li, S., Lu, L., Xie, J., Zhang, L., Wang, R., & Luo, X. (2013). The effectiveness of zinc proteinate for chicks fed a conventional corn-soybean meal diet. *The Journal of Applied Poultry Research*, *22*(3), 396-403. https://doi.org/10.3382/japr.2012-00564

Liu, Y., Qiu, N., Gao, D., & Ma, M. (2018). Comparative proteomic analysis of chicken, duck, and quail egg yolks. *International Journal of Food Properties*, *21*(1), 1311-1321. https://doi.org/10.1080/10942912.2018.1489836

Mohamed, L. A., El-Hindawy, M. M., Alagawany, M., Salah, A. S., & El-Sayed, S. A. A. (2019). Effect of low- or high-CP diet with cold-pressed oil supplementation on growth, immunity and antioxidant indices of growing quail. *Journal of Animal Physiology and Animal Nutrition*, *103*(5), 1380-1387. https://doi.org/10.1111/jpn.13121

Molino, A., Garcia, E., Santos, G., Filho, J. V., Baldo, G., & Paz, I. A. (2015). Photostimulation of Japanese quail. *Poultry Science*, *94*(2), 156–161. https://doi.org/10.3382/ps/peu039

Narinc, D., Uckardes, F., & Aslan, E. (2014). Egg production curve analyses in poultry science. *World Poultry Science Journal*, *70*(4), 817–828. https://doi.org/10.1017/S0043933914000877

Nasaka, J., Nizeyi, J. B., Okello, S., & Katongole, C. B. (2017). Characterization of feeding management practices of quails in urban areas of Uganda. *Journal of Animal and Veterinary Advances, 16*(8-12), 92-100, https://doi.org/10.3923/javaa.2017.92.100

Patra, A., & Lalhriatpuii, M. (2020). Progress and prospect of essential mineral nanoparticles in poultry nutrition and feeding - A review. *Biological Trace Element Research*, *197*(1), 233–253. https://doi.org/10.1007/s12011-019-01959-1

Reda, F. M., El-Saadony, M. T., El-Rayes, T. K., Attia, A. I., El-Sayed, S. A., Ahmed, S. Y., Madkour, M., & Alagawany, M. (2021). Use of biological nano zinc as a feed additive in quail nutrition: biosynthesis, antimicrobial activity and its effect on growth, feed utilisation, blood metabolites and intestinal microbiota. *Italian Journal of Animal Science, 20*(1), 324-335. https://doi.org/10.1080/1828051X.2021.1886001

Sahin, K., Smith, M., Onderci, M., Sahin, N., Gursu, M., & Kucuk, O. (2005). Supplementation of zinc from organic or inorganic source improves performance and antioxidant status of heat-distressed quail. *Poultry Science*, *84*(6), 882–887. https://doi.org/10.1093/ps/84.6.882

Singh, R. A., & Johari, D. C. (1977). *Japanese quail production in India*. Technical Bulletins of ICAR-CARI, Izatnagar, Bareilly.

Snedecor, G. W., & Cochran, W. G. (1994). *Statistical methods* (9th ed.). The Iowa State University Press.

Statistical Package for the Social Sciences SPSS. (2011). *Statistical Package for the Social Sciences (Version 20.0). SPSS Inc.* https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-20

Talapatra, S. K., Roy, S. C., & Sen, K. C. (1940). The analysis of mineral constituents in biological materials. I. Estimation of phosphorus, calcium, magnesium, sodium and potassium in food stuffs. *Indian Journal of Veterinary Science and Animal Husbandry*, 10, 243–58. https://www.cabdirect.org/cabdirect/abstract/19401403292

Received - 22.09.2025, Accepted - 13.11.2025, Published - 22.11.2025 (Online), 01.12.2025 (Print)
Section Editor: Prof. Guru Prasad Mandal, Invited Section Editor