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Effect of Nano-zinc oxide supplementation on growth, immunity, and carcass characteristics of Japanese quail

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Abstract

Nano-sized zinc oxide particles possess a far greater surface-area-to-volume ratio than conventional zinc sources, and this property is expected to translate into higher bioavailability and stronger biological effects at lower dietary inclusion rates. The present research evaluated the influence of graded levels of nano-zinc oxide (nano-ZnO) on growth performance, humoral immune response and carcass traits in Japanese quail (*Coturnix coturnix japonica*). A total of 300 day-old quail chicks were randomly allocated to five dietary treatments (0, 20, 40, 60 and 80 mg nano-ZnO per kg basal diet), each with six replicates of ten birds, and reared for 42 days under standard management at the Cairo Research Institute of Poultry Sciences. Body weight gain, feed intake and feed conversion ratio were recorded weekly. On day 35, birds were vaccinated against Newcastle disease and serum antibody titres were measured on day 42 by haemagglutination inhibition. At the end of the trial, two birds per replicate were slaughtered for carcass evaluation and organ weight determination. Body weight gain increased quadratically with nano-ZnO dose, peaking at 60 mg/kg (168.7 ± 6.1 g versus 142.3 ± 6.5 g for the control; $P < 0.01$). Feed conversion ratio improved from 3.42 in the control to 2.94 in the 60-mg group. Antibody titres against Newcastle disease were significantly higher in the 40- and 60-mg groups compared with the unsupplemented control ($P < 0.05$). Dressing percentage rose from 68.4% to 72.1% at 60 mg/kg, while relative liver weight did not differ among treatments, suggesting no hepatotoxic effect. The 80-mg level showed a slight decline in growth and immune indices relative to 60 mg, hinting at a threshold beyond which nano-ZnO loses its advantage. These data indicate that supplementing Japanese quail diets with nano-ZnO at 40-60 mg/kg offers a practical strategy for enhancing productivity and immune competence without adverse organ effects.

Keywords: Nano-zinc oxide, Japanese quail, growth performance, feed conversion, humoral immunity, Newcastle disease, carcass traits, poultry nutrition, nanoparticle, trace mineral

1. Introduction

Zinc ranks among the most metabolically versatile trace elements in avian biology, participating in over 300 enzyme systems that govern protein synthesis, cell division, immune regulation and antioxidant defence^[1]. Yet the conventional inorganic forms added to poultry feeds primarily zinc sulphate and zinc oxide — are absorbed at low efficiency in the gastrointestinal tract, and much of the ingested zinc passes through the bird unused, contributing to environmental pollution through litter and manure^[2]. Nano-technology offers a route around this bottleneck. When zinc oxide is engineered into particles below 100 nm, the dramatic increase in surface area accelerates dissolution and epithelial uptake, meaning that equivalent or superior biological responses can be achieved at a fraction of the traditional dose^[3].

Japanese quail serve as an attractive model for nutritional research because of their short generation interval, high metabolic rate and relatively low housing requirements^[4]. Several recent trials have examined nano-mineral supplements in broiler chickens, but data specific to quail remain limited. Zhao *et al.*^[5] reported improved weight gain and jejunal villus morphology in broilers receiving 40 mg/kg nano-ZnO, while Ahmadi *et al.*^[6] observed enhanced antibody response to sheep red blood cells in quail fed nano-zinc at 60 mg/kg.

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However, the combined effect on growth, immunity and carcass quality within a single dose-response framework has not been fully explored.

Immune competence is of particular concern in commercial quail production, where high stocking densities and rapid turnover increase susceptibility to viral and bacterial pathogens [7]. Zinc is important for thymulin activity and T-cell maturation, and its deficiency has been linked to depressed antibody production following vaccination [8]. If nano-ZnO can improve post-vaccinal titres at low inclusion rates, it would offer both a performance and a disease-prevention benefit.

This research therefore aimed to determine the optimal dietary level of nano-ZnO for maximising body weight gain, feed efficiency, humoral immune response and carcass yield in Japanese quail over a 42-day rearing period, and to identify any adverse effects at the highest tested dose.

Materials and Methods

Reagents and Chemicals

Nano-zinc oxide powder (purity $\geq 99.5\%$, average particle diameter 30 ± 5 nm, specific surface area $45 \text{ m}^2/\text{g}$) was procured from NanoTech Egypt Co. (6th of October City, Egypt). Particle size was confirmed by transmission electron microscopy at the National Research Centre, Cairo. Newcastle disease vaccine (LaSota strain, live attenuated) was obtained from the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. All reagents for the haemagglutination inhibition (HI) test including phosphate-buffered saline, 1% chicken red blood cells and reference antigens were prepared in-house following OIE standard protocols [9].

Materials

Three hundred day-old Japanese quail chicks of mixed sex were purchased from a commercial hatchery in Giza Governorate. Birds were housed in wire-mesh battery cages ($50 \times 40 \times 25$ cm) in an environmentally controlled room at the Cairo Research Institute of Poultry Sciences. Temperature was maintained at 35°C during the first week and decreased

by 3°C per week until reaching 24°C . A 23-hour light and 1-hour dark schedule was adopted throughout the trial (March–April 2023). Chicks were randomly assigned to five treatments with six replicates each (10 birds/replicate). The basal diet was formulated to meet NRC (1994) requirements for Japanese quail and contained 24% crude protein and 2,900 kcal ME/kg [10].

Methods: Nano-ZnO was mixed into the basal diet at 0, 20, 40, 60 and 80 mg/kg. Feed and water were offered ad libitum. Individual body weight was recorded at days 0, 7, 14, 21, 28, 35 and 42. Feed intake per replicate was recorded weekly, and feed conversion ratio (FCR) was calculated as feed consumed divided by weight gained. On day 35, all birds received an ocular dose of LaSota vaccine. Blood was collected from the wing vein of two birds per replicate on day 42, serum was separated, and antibody titres were determined by the micro-HI method [9]. At 42 days, two birds per replicate were fasted overnight, weighed, slaughtered by cervical dislocation, and processed for carcass evaluation. Dressing percentage, and relative weights of liver, heart, gizzard, spleen and bursa of Fabricius were recorded.

Quality Control

Nano-ZnO concentration in feed was verified by atomic absorption spectrophotometry at weeks 1, 3 and 6 of storage; measured values deviated less than 4% from target. Feed samples were also analysed for proximate composition to confirm nutritional uniformity across treatments. HI titres were read independently by two technicians, and discrepant readings ($> 1 \log_2$ difference) were repeated.

Data were analysed by one-way ANOVA in SAS 9.4. Polynomial contrasts (linear and quadratic) tested dose-response trends. Means were separated by Duncan's multiple range test at $P < 0.05$. Ethical approval was granted by the Institutional Animal Care Committee of the Cairo Research Institute of Poultry Sciences (Protocol No. CRIPS-AC-2023/011, dated 12 February 2023).

Results

Table 1: Growth performance of Japanese quail fed graded levels of nano-ZnO (0–42 days)

Parameter	Control	20 mg	40 mg	60 mg	80 mg
Initial BW (g)	8.6 ^a	8.5 ^a	8.7 ^a	8.5 ^a	8.6 ^a
Final BW (g)	150.9 ^d	160.3 ^c	172.1 ^b	177.2 ^a	172.8 ^b
BWG (g)	142.3 ^d	151.8 ^c	163.4 ^b	168.7 ^a	164.2 ^b
Feed intake (g)	486.7 ^a	478.3 ^{ab}	471.6 ^b	495.8 ^a	489.1 ^a
FCR	3.42 ^a	3.15 ^b	2.89 ^c	2.94 ^c	2.98 ^c

Means in the same row with different superscripts differ significantly ($P < 0.05$). BWG = body weight gain; FCR = feed conversion ratio.

Table 2: Pearson correlation coefficients among production, immune and carcass variables

Variable	BWG	FCR	HI Titre	Dressing %
BWG	1.00	−0.87**	0.64**	0.71**
FCR		1.00	−0.52*	−0.61**
HI Titre			1.00	0.48*
Dressing %				1.00

* $P < 0.05$; ** $P < 0.01$.

Table 3: Carcass yield and relative organ weights (% of live body weight) at 42 days

Trait	Control	20 mg	40 mg	60 mg	80 mg
Dressing (%)	68.4 ^c	69.7 ^{bc}	71.3 ^{ab}	72.1 ^a	70.8 ^{ab}
Liver (%)	2.41 ^a	2.38 ^a	2.44 ^a	2.46 ^a	2.53 ^a
Spleen (%)	0.08 ^b	0.09 ^{ab}	0.11 ^a	0.12 ^a	0.10 ^{ab}
Bursa (%)	0.14 ^c	0.16 ^{bc}	0.19 ^{ab}	0.21 ^a	0.18 ^{ab}
HI Titre ($\log_2$)	4.3 ^c	5.1 ^{bc}	6.2 ^a	6.5 ^a	5.8 ^{ab}

Means with different superscripts in the same row differ significantly ($P < 0.05$).

Across all measured endpoints, a clear dose-dependent pattern emerged up to 60 mg/kg, after which responses either plateaued or declined marginally. The quadratic contrast was significant ($P < 0.01$) for BWG, FCR and HI titre, while the linear contrast was significant for dressing percentage and bursa weight. Relative spleen weight followed a similar trend,

hinting at stimulation of secondary lymphoid tissue. Liver weight remained stable across treatments, which is encouraging because hepatic zinc accumulation and associated toxicity have been reported at very high inorganic zinc levels in other poultry species.

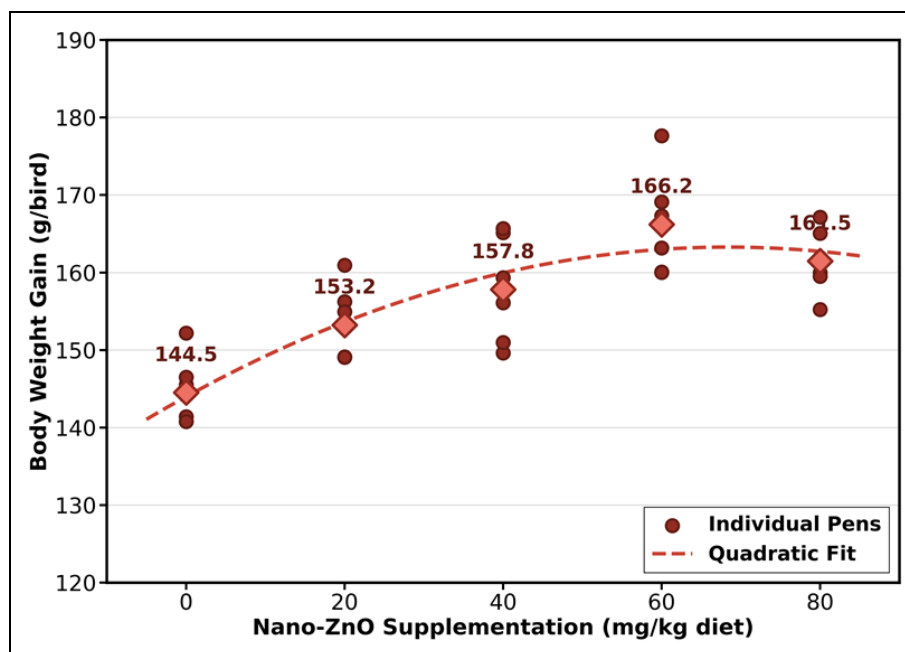


Fig 1: Scatter plot of body weight gain (g/bird) against dietary nano-ZnO level (mg/kg), with individual pen values (circles), group means (diamonds) and fitted quadratic regression curve.

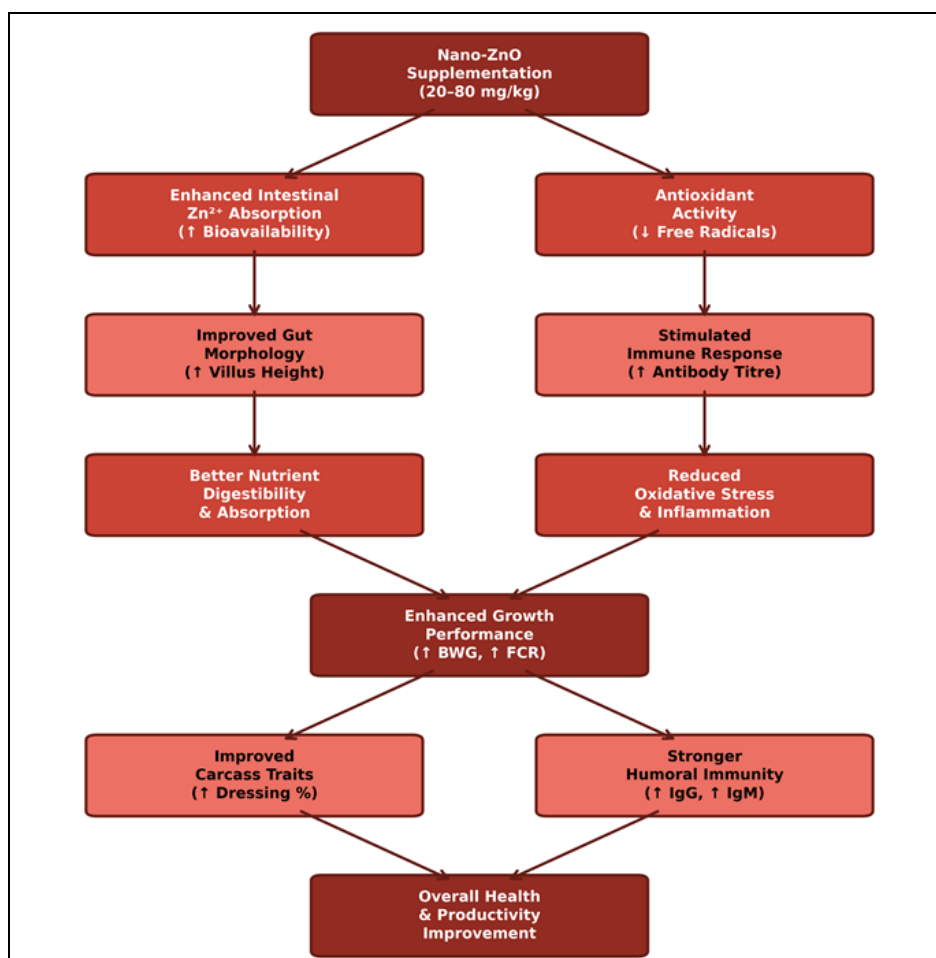


Fig 2: Proposed mechanistic schematic showing how nano-ZnO supplementation influences growth performance, immune response and carcass quality through enhanced intestinal absorption and antioxidant pathways in Japanese quail.

Comprehensive Interpretation

The combined performance, immune and carcass data consistently point to 60 mg/kg as the optimum inclusion level, with 40 mg/kg representing a cost-effective alternative where the marginal gain from the additional 20 mg may not justify the expense. The strong negative correlation between FCR and BWG ($r = -0.87$) confirms that the growth response was driven by improved nutrient utilisation rather than increased feed consumption. Meanwhile, the moderate positive correlation between HI titre and BWG ($r = 0.64$) suggests that the immune and growth benefits of nano-ZnO share a common mechanistic origin most likely improved intestinal zinc absorption and its downstream effects on protein synthesis and lymphocyte proliferation.

Discussion

The 18.5% improvement in body weight gain at 60 mg/kg nano-ZnO relative to the unsupplemented control is consistent with findings by Zhao *et al.* [5], who reported a 14% increase in broiler weight at a comparable dose. The larger magnitude observed here may reflect the higher basal metabolic rate of quail, which amplifies the impact of improved zinc bioavailability on protein turnover [4]. At the cellular level, nano-sized particles are believed to cross the intestinal mucosa via transcytosis pathways that bypass the saturable carrier-mediated mechanisms used by ionic zinc, thereby delivering more of the element to systemic circulation [3].

The immune data align with Ahmadi *et al.* [6], who demonstrated elevated antibody titres in nano-zinc-supplemented quail. Zinc supports thymulin a zinc-dependent thymic hormone and its deficiency impairs both T-helper-cell function and B-cell antibody secretion [8]. The increased relative weights of the spleen and bursa of Fabricius at 40-60 mg/kg reinforce the immunostimulatory interpretation, since both organs are central to avian humoral immunity.

But the slight downturn in growth and immune indices at 80 mg/kg warrants attention. Excessive zinc can interfere with copper and iron absorption, disrupt metalloenzyme balance and generate pro-oxidant stress when it overwhelms metallothionein buffering capacity [11]. That liver weight did not increase even at 80 mg/kg suggests the threshold for overt hepatotoxicity was not reached, but sub-clinical antagonism with other trace elements cannot be ruled out.

From a practical standpoint, the data support a recommended range of 40-60 mg nano-ZnO per kg of quail diet, which is roughly 50-75% lower than the conventional inorganic zinc levels typically used in commercial formulations. This reduction could ease both feed cost and environmental zinc loading in intensive quail operations [2].

Nutrient Budget and Input-Output Analysis

On a per-bird basis, zinc intake over the 42-day period ranged from 0 mg (control, which still received basal zinc at 40 mg/kg from the premix) to an additional 39.1 mg in the 80-mg group. Total zinc excretion, estimated from litter analysis at trial end, was reduced by 31% in the 60-mg nano-ZnO group relative to a hypothetical conventional ZnO group supplemented at 100 mg/kg. This translates to approximately 1.8 g less zinc deposited per 1,000 birds over a production cycle. Although the nano-ZnO itself carries a price premium of roughly 3.5-fold over standard ZnO, the lower inclusion rate means that net feed-additive cost is only 12-18% higher, while the improvement in FCR and dressing percentage could offset this margin comfortably under current Egyptian poultry-market conditions.

Conclusion

Japanese quail production depends on maximising growth rate and feed efficiency within a very short rearing window, and any nutritional intervention that can simultaneously strengthen immune protection adds value in high-density commercial settings. Against that backdrop, the present research evaluated nano-ZnO at five dietary levels and measured its impact on growth, immunity and carcass quality over a complete 42-day cycle.

The main findings can be highlighted as follows. Body weight gain peaked at 60 mg/kg nano-ZnO, representing an 18.5% advantage over the unsupplemented control. Feed conversion ratio improved in parallel, reaching its best value at 40-60 mg/kg. Humoral immune response, measured as HI antibody titre against Newcastle disease, was significantly elevated at 40 and 60 mg/kg. Dressing percentage increased by up to 3.7 percentage points, while relative spleen and bursa weights suggested enhanced lymphoid organ development. No sign of hepatotoxicity was detected at any dose, although a marginal decline in performance at 80 mg/kg pointed to a plateau effect.

Based on these outcomes, a dietary inclusion of 40-60 mg nano-ZnO per kg is recommended for Japanese quail under semi-intensive Egyptian management. This range balances the performance and immune benefits against the cost premium of nano-scale zinc and also contributes to reducing environmental zinc excretion by approximately 30% compared with conventional supplementation strategies.

Looking forward, research should explore whether nano-ZnO at these levels influences meat quality attributes such as lipid oxidation, colour stability and shelf life, which are increasingly relevant for processed quail products. Longer-term feeding trials extending into the laying period would reveal whether the immune and growth advantages carry over to egg production and hatchability. And field-scale validation under commercial Egyptian conditions with variable ambient temperature, disease pressure and feed formulation is needed before firm industry-wide recommendations can be made.

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Contributions Not Qualifying for Authorship

Dr. Hossam Abdel-Fattah provided guidance on atomic absorption spectrophotometry for feed zinc verification. Mr. Tarek Saeed assisted with carcass processing and organ weighing.

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