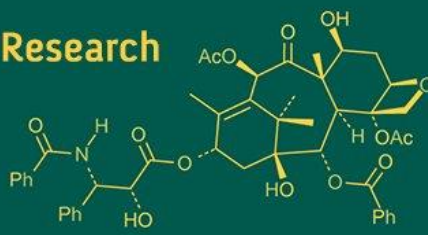


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Effects of dietary zinc oxide nanoparticles on immune response and antioxidant status in broiler chicks

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Abstract

The present study aimed to investigate the effect of zinc oxide nanoparticles (ZnONPs) on immune response and antioxidant status. A total of 150 one-day old straight run broiler chicks were randomly allotted in 5 dietary treatments fed a basal diet supplemented with 80 mg/kg zinc oxide (control group), 60 mg/kg zinc methionine (organic zinc) and 60 or 40 or 20 mg/kg ZnONPs respectively, for 35 days. Compared with control group, different levels of ZnONPs did not alter antibody titer against Newcastle disease virus (NDV) and Infectious Bursal Disease virus (IBDV). However, supplementation of 20 mg/kg ZnONPs significantly downregulated the mRNA expressions of IFN γ , IL-6, and IL-10 genes and upregulated the *BF2* gene compared to the control group. Moreover, 20 mg/kg ZnONPs significantly upregulated the mRNA expression of *superoxide dismutase (SOD)*, *glutathione peroxidase (GPx)* and *catalase (CAT)* genes in liver and breast muscle tissues and significantly reduced malondialdehyde (MDA) concentration in the liver tissue compared to control group. Supplementation of ZnONPs at a lower level (20 mg/kg feed) to the broilers enhanced antioxidant activity and immune response compared with conventional sources of zinc.

Keywords: Antioxidant activity, Broiler, Immune response, mRNA expression and ZnONPs

Introduction

The poultry sector represents one of the most dynamic components of modern agriculture because of its rapid production cycle and substantial economic contribution. It plays a crucial role in supplying eggs, meat, and various processed poultry products to meet the increasing nutritional demands of the growing population (Saber *et al.*, 2026) ^[1]. However, the sector encounters several nutritional and health-related challenges, particularly deficiencies of essential micronutrients such as zinc which, although required in small quantities, are important for health, growth, and immune function of the poultry (Al-Jaf *et al.*, 2025) ^[2]. It participates in numerous biochemical and physiological processes and functions as an antioxidant by scavenging the free radicals. In addition, zinc contributes to bone formation, enzyme structure, growth performance, and immunity. It also plays a critical role in the metabolism of proteins, lipids, and carbohydrates (El-Ratel *et al.*, 2025) ^[3].

Despite its importance, zinc lacks a specialized storage mechanism in the body, necessitating a continuous dietary supply to meet metabolic demands (Gatti *et al.*, 2026) ^[4]. To ensure adequate levels, zinc is commonly added to poultry diets in inorganic forms, such as zinc oxide (ZnO), primarily because of their lower cost compared with organic sources (Zhao *et al.*, 2014) ^[5]. However, inorganic zinc may interact with phytate in feed, reducing its bioavailability and necessitating higher supplementation levels. This can increase production costs and may lead to environmental concerns due to excess mineral excretion. (Samy *et al.*, 2025) ^[6]. Although chelated or organic forms of zinc exhibit greater bioavailability than inorganic sources, their use in poultry diets is often limited by their higher price (Saber *et al.*, 2026) ^[1].

In this sense, nanotechnology enables the production of zinc oxide nanoparticles (ZnO NPs) with extremely small particle sizes ranging from 1 to 100 nm. These nanoparticles can enhance the bioavailability of zinc and intestinal absorption, thereby enhancing the mineral utilization efficiency while reducing excessive mineral excretion (Mohd Yusof *et al.*, 2023) ^[7]. Such innovations could provide effective nutritional strategies to overcome persistent challenges in intensive poultry production systems, where oxidative stress and immune

suppression remain major constraints affecting optimal productivity (Al-Jaf *et al.*, 2025) [2]. Due to a lack of knowledge on the optimal inclusion level of ZnONPs in broiler chicken diets, the purpose of this study is to investigate the effect of various levels of ZnONPs supplementation on humoral immunity, cell-mediated immune response, and antioxidant defence.

Materials and Methods

Synthesis and characterization of ZnONPs

ZnONPs were synthesized using the planetary ball milling technique following the method described by Mozhi *et al.* (2024) [8]. The average particle size and zeta potential value of the synthesized ZnONPs were 7.7 nm and -31.9 mV, respectively, as determined using a particle size analyzer (Figures 1). High-Resolution Transmission Electron Microscopy further confirmed the presence of nearly hexagonal-shaped nanoparticles (Figure 2).

Birds and design of experiment

The experiment was carried out in an environmentally controlled poultry shed at Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai – 7. A total of 150 one-day-old broiler chicks (Vencobb 400) were randomly distributed into five dietary treatment groups, each with three replicates consisting of ten chicks per replicate. The birds were reared in a deep litter system for a period of five weeks. The dietary treatments consisted of different zinc sources added per kg of maize-soybean meal based basal diet as follows: T₁ (control group) – 80 mg/kg inorganic zinc (ZnO); T₂ – Zn-Met group containing 60 mg/kg organic zinc (zinc methionine); T₃, T₄ and T₅ – ZnONPs groups supplemented with zinc oxide nanoparticles at 60 mg/kg, 40 mg/kg and 20 mg/kg, respectively. The basal diets for the broiler were formulated according to the specifications of the Bureau of Indian Standards (BIS, 2007) [9]. The experimental birds were provided ad libitum access to feed and water, and a lighting program of 23 hours of light per day was maintained throughout the experimental period. The vaccination schedule followed during the study is presented in Table 1.

Measurements

Serum antibody titers against Newcastle disease virus and infectious bursal disease virus

To evaluate the antibody titers against NDV and IBDV in serum, blood samples were collected from the jugular vein of six birds prior to the vaccination from each treatment on days 21 and 35. The antibody titers were analysed using NDV and IBD Ab Test kits (IDEXX laboratories, USA) respectively as per the manufacturer's instructions by ELISA.

Lipid peroxidation assay

Immediately after sacrificing the birds on day 35, the liver tissues collected from six birds from each treatment were excised and washed in ice-cold normal saline. Liver tissues were crushed in a tissue homogenizer and 10% w/v homogenate was prepared with 0.05 M PBS (pH 7.4). This was used for the estimation of malondialdehyde (MDA). Lipid peroxide levels in the homogenates were determined as thiobarbituric acid reactive substances by the method of Ohkawa *et al.* (1979) [10]. Lipid peroxide levels were

expressed in nmol MDA/ mg of tissue.

mRNA expression of immune genes and antioxidant enzymes genes by quantitative real-time PCR

For the cell-mediated immune response-related genes (IFN- γ , IL-10, IL-6 and BF2) expression studies, 4 ml of whole blood was collected from six birds in each treatment group for the isolation of peripheral blood mononuclear cells (PBMCs) using the density gradient centrifugation method (Kaiser *et al.*, 2006) [11]. In addition, liver and muscle tissue samples were obtained from six birds per treatment at the time of slaughter to study the expression of antioxidant enzyme genes (SOD, GPx and CAT). Total RNA from PBMCs, liver and muscle tissues was extracted using the TRIzol method following the protocol described by Rio *et al.* (2010) [12]. The concentration and purity of the extracted RNA were measured using a NanoDrop spectrophotometer (Thermo Scientific NanoDrop™ One Microvolume UV-Vis Spectrophotometer). Complementary DNA (cDNA) was synthesized from the isolated RNA using the iScript™ cDNA synthesis kit (Bio-Rad). Quantitative real-time PCR was carried out using a Bio-Rad CFX Opus 96 Real-Time PCR system with iTaq Universal SYBR Green Supermix (Bio-Rad). The specific primer sequences and thermal cycling conditions used for the analysis are provided in Tables 2 and 3, respectively. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method described by Livak *et al.* (2001) [13] and normalized to 16s rRNA, which served as the housekeeping gene.

Statistical Analysis

The experimental data were analyzed by one-way ANOVA using SPSS v.20.0 statistical package and means of different treatments were computed using Duncan's multiple range test (Duncan, 1995) [14]. The obtained results were expressed as mean $\pm$ standard deviation and $p < 0.05$ was considered as statistically significant.

Results

1. Immunological parameters

1.1. Humoral Immune Response

No significant ($p > 0.05$) difference was observed among the dietary treatments on the serum antibody titers against NDV and IBDV on days 7, 21, and 35 in broiler chicks (Table 4).

1.2. Cell-mediated immune response

Gene expression analysis of cytokines

Supplementation of broiler chick feed with the lowest dose of ZnONPs (20 mg/kg) significantly ($p < 0.05$) downregulated the mRNA expressions of pro-inflammatory cytokines viz., IFN γ and IL-6 genes and anti-inflammatory cytokine (IL-10) gene compared to the control group (Figure 3a). However, no significant ($p > 0.05$) difference was observed in the mRNA expression of IFN γ and IL-10 genes among birds fed on 20, 40 and 60 mg/kg ZnONPs and 60 mg/kg of Zinc methionine.

Gene expression analysis of MHC class I molecule

Dietary supplementation of lower doses of ZnONPs (20 and 40 mg/kg) significantly ($p < 0.05$) upregulated (3.63 and 2.89-fold respectively) the MHC class I molecule (BF2 gene) gene expression as compared with control and Zn-Met groups (Figure 3b).

2. Antioxidant Status

Lipid Peroxidation

The malondialdehyde (MDA) concentration in the liver tissue of birds fed on 20 mg/kg of ZnONPs was significantly lower ($p < 0.05$) than control and Zn-Met group, but no significant changes were observed among various levels (20, 40 and 60 mg/kg) of ZnONPs (Table 5). Moreover, MDA concentration in the liver was linearly reduced as dietary levels of ZnONPs decreased.

Gene expression analysis of antioxidant enzymes in liver

Compared to control and zinc methionine, the addition of ZnONPs (20 – 60 mg/kg) in the broiler chicks' diet significantly ($p < 0.05$) upregulated the mRNA expression of *SOD*, *CAT* and *GPx* in the liver (Figure 4a). Furthermore, a declining trend in mRNA expression of these antioxidant enzymes in liver tissue was observed as the level of ZnONPs increases in the diet, while the highest level of upregulation of genes was observed in the lowest dose of ZnONPs (20 mg/kg diet).

Gene expression analysis of antioxidant enzymes in muscle

The inclusion of ZnONPs (20 – 60 mg/kg) broiler chicks' diet significantly ($p < 0.05$) upregulated the mRNA expression of *SOD* and *CAT* in the breast muscle tissue, while the gene expression of *GPx* in breast muscle tissue was significantly ($p < 0.05$) higher at 20 mg/kg ZnONPs and 60 mg/kg zinc methionine-fed group as compared with other groups. Moreover, as the level of ZnONPs increases in the diet, declining trend in mRNA expression of these antioxidant enzymes were observed and in the lowest dose of ZnONPs (20 mg/kg diet), the highest level of upregulation of genes was observed (Figure 4b).

Discussion

The present study revealed that dietary ZnONPs (20,40 and 60 mg/kg) had no effects on serum antibody titers against NDV and IBDV as compared with 80 mg/kg ZnO and 60 mg/kg zinc methionine, suggesting that even 20 mg/kg of ZnONPs might also deliver the required zinc for humoral immune response as provided by dietary 80 mg/kg ZnO and 60 mg/kg zinc methionine in broiler chicks. In agreement with the present results, other researchers indicated no effect of different zinc sources on antibody titers against NDV and IBDV (Manangi *et al.*, 2012; Moghaddam *et al.*, 2009)^[15,16]. Previous studies have consistently reported noteworthy effects of ZnONPs on antibody titers against Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV) in broiler chicks (Al-Shemmari and Fahad, 2019; Tian *et al.*, 2010)^[17,18].

Concerning the cytokines expression, our results showed a significant ($p < 0.05$) downregulation of the pro-inflammatory cytokines viz., IFN γ and IL-6 genes in the broiler chicks supplemented with 20 mg/kg ZnONPs compared to the control group which was consistent with results reported in the jejunum of pigs (Hu *et al.*, 2013)^[19].

These results imply that ZnONPs have an obvious effect on cell-mediated immune response in broiler chicks. Accordingly, Zhang *et al.* (2022)^[20] reported that 40 or 80 mg/kg ZnONPs significantly decreased the mRNA abundance of IFN γ in the jejunal mucosa of broiler chickens. The organic zinc and different levels of ZnONPs (20, 40, and 60 mg/kg) significantly ($p < 0.05$) downregulated the expression of the IL-10 gene. On the contrary, Zhang *et al.* (2022)^[20] indicated that serum concentration of IL-10 increased linearly with the increasing level of dietary ZnONPs (40 – 160 mg/kg diet). The current study showed the significant ($p < 0.05$) upregulation of the BF2 gene in birds supplemented with either 20 or 40 mg/kg of ZnONPs. Moreover, supplementation of low levels (20 or 40 mg/kg) of ZnONPs was more efficient than high levels (Attia *et al.*, 2020)^[21]. Therefore, downregulation of IFN- γ , IL-6, and IL-10 with upregulation of BF2 indicates increasing the bioavailability of Zn as in the ZnONPs, might support the enhancement of immune response to broilers. Malondialdehyde (MDA) is an important index for lipid peroxidation (Nielsen *et al.*, 1997)^[22] and lipid peroxidation is an indication of oxidative damage to the cells (Hafez *et al.*, 2020)^[23]. Zinc is known as an effective antioxidant trace mineral in the biological system (Dukare *et al.*, 2021)^[24]. In some studies, Attia *et al.* (2020)^[21]; Eskandani *et al.* (2021)^[25]; as well as the results of the present study, indicated that MDA content in liver tissue was affected by different zinc sources, and the significantly ($p < 0.05$) lowest MDA content was observed in 20 mg/kg of ZnONPs group. This finding confirmed the positive effect of ZnONPs even at lower doses against lipid peroxidation and thereby enhancing the antioxidant defense of the broiler chicks.

The enzymatic antioxidative stress responses were reported to be effective only in the presence of sufficient amounts of cofactors such as Zn in the organism (Underwood and Suttle, 2001)^[26]. Zinc acts as an activator of *SOD* (Qu *et al.*, 2023)^[27] and *SOD* catalyzes superoxide radicals into H₂O₂ and it is reduced to water by *GPx* or *catalase* (Surai *et al.*, 2016)^[28]. In this study, the antioxidant property of ZnONPs has been confirmed even at the molecular level as reflected by the significant upregulation of the *SOD*, *CAT* and *GPx* genes in the liver and breast muscle tissues of the broiler chicks. The results of the present study are in agreement with Zhao *et al.* (2014)^[5] and Fathi *et al.* (2016)^[29] who suggested that a lower concentration of Zn (20 mg/kg) consistently stimulates antioxidative function in broilers by improving the *Cu-Zn-SOD* activity and catalase activity, while a higher concentration of nano zinc (40 mg/kg) was not associated with a significant increase in the *SOD* activity in the serum.

In conclusion, supplementation of ZnONPs even at a lower level (20 mg/kg feed) exhibited superior effects compared to inorganic and organic sources of zinc in terms of enhancing cell-mediated immunity by regulating inflammatory cytokine activity and fortifying antioxidant defense by reducing oxidative stress of broilers.

Table 1: Vaccination Schedule

Age	Name of the Vaccine	Route of Administration
0 day	Marek's disease vaccine	Subcutaneous
7 th day	Newcastle disease vaccine – RDV- F strain	Intra ocular
14 th day	Infectious Bursal Disease vaccine (Georgia strain)	Intra ocular
21 st day	Newcastle disease vaccine - Lasota strain	Oral

Table 2: The sense and antisense primer sequences of genes used for Real-Time PCR study

Gene	Orientation	Primers sequences (5' - 3')	Product size (bp)	Reference
16S	Forward	GTAACGCAAGCGATCNCG	130	Xie <i>et al.</i> , (2020) ^[30]
	Reverse	AACCGCGACGCTTTCCAA		
IFN- γ	Forward	ATCATACTGAGCCAGATTGTTTCG	140	
	Reverse	TCTTTCACCTTCTTCACGCCAT		
IL-10	Forward	CACGCGGAGGGCGTTAAA	186	
	Reverse	CAGGTGAAAGTCAGCCCGT		
IL-6	Forward	CAACCTCAACCTGCCCAA	86	El-Bahr <i>et al.</i> , (2020) ^[31]
	Reverse	GGAGAGCTTCCTCAGGCATT		
BF2	Forward	CCGTGGTTCGTGACTGTGGGGTAC	222	O'Neill <i>et al.</i> (2009) ^[32]
	Reverse	CACTGTGTGAGACCCGCCGGT		
SOD	Forward	TTCCTGACCTGCCTTACGACTAT	138	Li <i>et al.</i> (2011) ^[33]
	Reverse	CCAGCGCCTCTTTGTATTCT		
CAT	Forward	ACTGGTGCTGGCAACCC	86	Rimoldi <i>et al.</i> (2015) ^[34]
	Reverse	ACGTGGCCCAACTGTCAT		
GPx	Forward	AACCAATTCGGGCACCAG	122	Yuan <i>et al.</i> (2012) ^[35]
	Reverse	CCGTTCACCTCGCACTTCTC		

Table 3: Thermal cyclic conditions used for Real-Time PCR study

Thermal cyclic conditions	Genes		
	IFN γ , IL-6 & IL-10	SOD & GPx	CAT & BF-2
Initial denaturation temperature	95 °C for 3 minutes	95 °C for 3 minutes	95 °C for 5 minutes
Final denaturation temperature	95 °C for 30 seconds	95 °C for 30 seconds	92 °C for 20 seconds
Annealing temperature	60 °C for 15 seconds	60 °C for 30 seconds	60 °C for 1 minute
Extension temperature	72 °C for 35 seconds	72 °C for 45 seconds	72 °C for 1 minute
Number of Cycles	40	40	40
Melting curve	65 °C – 95 °C	65 °C – 95 °C	65 °C – 95 °C

Table 4: Effects of ZnONPs on serum antibody levels of NDV and IBDV of broilers (Mean $\pm$ SD)

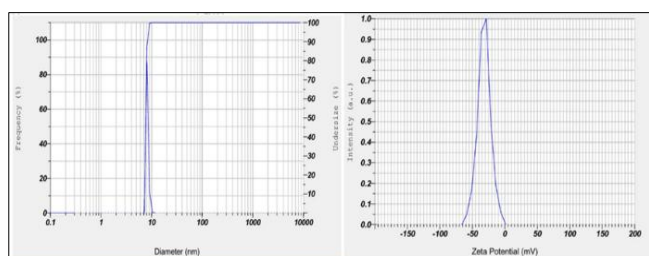
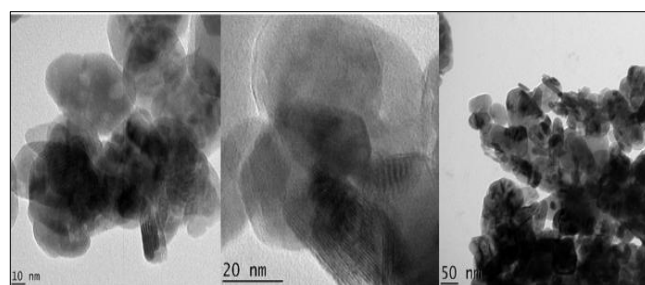
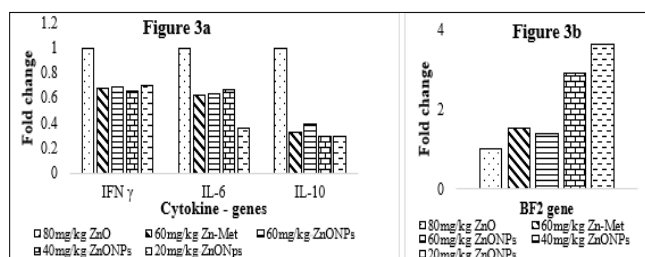
Type	Growth Stage	Treatments					P value
		T ₁	T ₂	T ₃	T ₄	T ₅	
IBDV	Day 21	1.97 $\pm$ 0.08	1.88 $\pm$ 0.22	1.94 $\pm$ 0.16	2.00 $\pm$ 0.12	2.04 $\pm$ 0.15	0.75
	Day 35	2.35 $\pm$ 0.53	2.28 $\pm$ 0.71	2.01 $\pm$ 0.36	2.19 $\pm$ 0.25	2.41 $\pm$ 0.70	0.63
NDV	Day 21	2.78 $\pm$ 0.19	2.51 $\pm$ 0.18	2.72 $\pm$ 0.18	2.82 $\pm$ 0.28	2.75 $\pm$ 0.27	0.27
	Day 35	2.37 $\pm$ 0.42	2.14 $\pm$ 0.23	2.72 $\pm$ 0.56	2.53 $\pm$ 0.57	2.43 $\pm$ 0.37	0.36

[#]Each value represents the mean of six observations. T₁ – 80 mg/kg ZnO; T₂ – Zn met-60 mg/kg; T₃ – ZnONPs- 60mg/kg; T₄ – ZnONPs - 40 mg/kg; T₅ – ZnONPs - 20 mg/kg

Table 5: Effects of supplementation of ZnONPs on the concentration of malondialdehyde in the liver of broilers (Mean $\pm$ SD)

Treatment	MDA (nmol /g protein)
80 mg/kg ZnO	3.35 ^b $\pm$ 1.24
60 mg/kg Zn-Met	2.95 ^b $\pm$ 1.02
60 mg/kg ZnONPs	2.40 ^{ab} $\pm$ 1.22
40 mg/kg ZnONPs	2.12 ^{ab} $\pm$ 1.17
20 mg/kg ZnONPs	1.44 ^a $\pm$ 0.80
P value	0.05

[#]Each value represents the mean of six observations. Mean bearing different superscripts in a column differ significantly with ($p < 0.05$).

**Fig 1:** Particle size and Zeta potential analysis of ZnONPs**Fig 2:** Transmission electron microscopic image of ZnONPs**Fig 3:** Effect of ZnONPs on relative mRNA expression of cytokine and BF2 genes of broilers

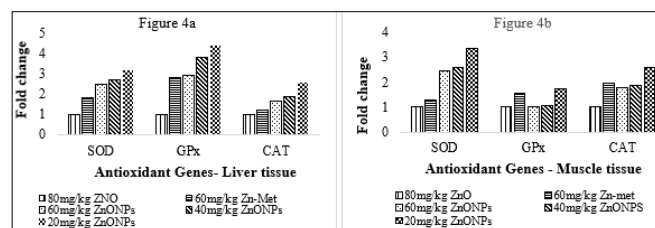


Fig 4: Effects of ZnONPs on relative mRNA expression of antioxidant enzymes in liver and muscle tissues of broilers

Abbreviations:

BIS-Bureau of Indian Standards; CAT-catalase; cDNA-complementary Deoxy Ribo Nucleic Acid; GPx- glutathione peroxidase; IBDV- Infectious Bursal Disease Virus; IFN – interferon; IL- interleukin; MDA- malondialdehyde; mg/kg – milligram per kilogram; MHC – major histocompatibility complex; mRNA-messenger Ribo Nucleic Acid; NDV-Newcastle Disease Virus; PBMC – peripheral blood mononuclear cells; PCR- Polymerase chain reaction; ROS – reactive oxygen species; SOD- superoxide dismutase; ZnO – zinc oxide; ZnONPs – zinc oxide nanoparticles; Zn-zinc;

Conflict of Interest

There is no conflict of interest among the authors.

Data availability statement

Data of research will be made available with the corresponding author on demand

Ethical statement

The experimental procedures in the study were approved by the Institutional Animal Ethical Committee (IAEC) at Madras Veterinary College, TANUVAS, Chennai. (L.No. 508/DFBS/IAEC/2022 dated on 05.05.2022).

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References

1. Saber HS, Alian HA. Nano zinc oxide improves growth rate, carcass traits, meat chemical composition, serum and tissue mineral profiles, mineral retention, and intestinal morphology in broiler chickens compared to inorganic and organic zinc. *Biol Trace Elem Res.* 2026;204(2):909-924.
2. Al-Jaf HAK, Rashid SA. Synergistic effects of zinc oxide and selenium nanoparticles in drinking water on broiler chicken growth, antioxidant, and immunity responses. *Vet Anim Sci.* 2025;100528.
3. El-Ratel IT, Khaled HE, Soma ME, Sara FF, Abdel-Khalek E, Hassan MA, *et al.* The synergetic effect of selenium or zinc oxide nanoparticles with chromium on mitigating thermal stress for sustainable production and improving antioxidant capacity and inflammatory cytokines of growing rabbits. *Arch Anim Breed.* 2025;68(1):43-55.
4. Gatti BS, Bacchi MA, Fernandes MA, Scotton KC, de Nadai Fernandes EA, Abdalla AL, *et al.* Kinetics of ⁶⁵Zn in sheep supplemented with micro- and nanoparticles of zinc oxide. *Biol Trace Elem Res.* 2026;204(2):812-819.
5. Zhao CY, Tan SX, Xiao XY, Qiu XS, Pan JQ, Tang ZX. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. *Biol Trace Elem Res.* 2014;160:361-367.
6. Samy A, Abd-Elsamee MO, Ezzelarab SM, Kabary HA, Elsherif HM. The impact of nano zinc on broiler productivity, tibia bone characteristics, and gut microbiota. *Sci Rep.* 2025;15:34107.
7. Mohd Yusof H, Abdul Rahman N, Mohamad R, Hasanah Zaidan U, Aiman Arshad M, Asmara Samsudin A. Effects of dietary zinc oxide nanoparticles supplementation on broiler growth performance, zinc retention, liver health status, and gastrointestinal microbial load. *J Trace Elem Miner.* 2023;4:100072.
8. Mozhiarasi V, Karunakaran R, Raja P, Radhakrishnan L. Effects of zinc oxide nanoparticles supplementation on growth performance, meat quality and serum biochemical parameters in broiler chicks. *Biol Trace Elem Res.* 2024;202(4):1683-1698.
9. Bureau of Indian Standards. Indian standards for poultry feeds – specifications. 5th ed. New Delhi: BIS; 2007.
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95(2):351-358.
11. Kaiser MG, Cheeseman JH, Kaiser P, Lamont SJ. Cytokine expression in chicken peripheral blood mononuclear cells after *in vitro* exposure to Salmonella enterica serovar Enteritidis. *Poult Sci.* 2006;85(11):1907-1911.
12. Rio DC, Ares M, Hannon GJ, Nilsen TW. Purification of RNA using TRIzol (TRI reagent). *Cold Spring Harb Protoc.* 2010;6.
13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods.* 2001;25(4):402-408.
14. Duncan DB. Multiple range and multiple F tests. *Biometrics.* 1955;11:1-42.
15. Manangi MK, Vazquez-Anon M, Richards JD, Carter S, Buresh RE, Christensen KD. Impact of feeding lower levels of chelated trace minerals versus industry levels of inorganic trace minerals on broiler performance, yield, footpad health, and litter mineral concentration. *J Appl Poult Res.* 2012;21(4):881-890.
16. Moghaddam HN, Jahanian R. Immunological responses of broiler chicks can be modulated by dietary supplementation of zinc-methionine in place of inorganic zinc sources. *Asian-Australas J Anim Sci.* 2009;22(3):396-403.
17. Al-Shemmari WH, Fahad MA. Immune response with some blood parameters of broiler chicks fed on rations containing different levels of zinc and folic acid. *Euphrates J Agric Sci.* 2019;11(2):32-38.
18. Tian YB, Zhou JR, Li QH, Jia JJ, Huang RH. Effects of Atractylodes polysaccharide on growth performance and serum biochemical parameters in piglets. *Chin J Anim Sci.* 2009;45:45-48.
19. Hu C, Song J, Li Y, Luan Z, Zhu K. Diosmectite–zinc oxide composite improves intestinal barrier function, modulates expression of pro-inflammatory cytokines and tight junction protein in early weaned pigs. *Br J Nutr.* 2013;110(4):681-688.
20. Zhang J, Yu C, Li Z, Li J, Chen Y, Wang T, *et al.* Effects of zinc oxide nanoparticles on growth, intestinal

- barrier, oxidative status and mineral deposition in 21-day-old broiler chicks. *Biol Trace Elem Res.* 2022;200(4):1826-1834.
21. Attia FM. Efficacy of dietary zinc oxide nanoparticles supplementation on serum biochemical, nutrients retention and chemical composition of meat and tibia in broiler chickens. *Egypt Poult Sci J.* 2020;40(1):29-46.
 22. Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of lifestyle factors. *Clin Chem.* 1997;43(7):1209-1214.
 23. Hafez A, Nassef E, Fahmy M, Elsabagh M, Bakr A, Hegazi E. Impact of dietary nano-zinc oxide on immune response and antioxidant defense of broiler chickens. *Environ Sci Pollut Res.* 2020;27:19108-19114.
 24. Dukare S, Mir NA, Mandal AB, Dev K, Begum J, Rokade JJ, *et al.* A comparative study on the antioxidant status, meat quality, and mineral deposition in broiler chicken fed dietary nano zinc *viz-a-viz* inorganic zinc. *J Food Sci Technol.* 2021;58:834-843.
 25. Eskandani M, Janmohammadi H, Mirghelenj SA, Ebrahimi M, Kalanaky S. Effects of zinc nanoparticles on growth performance, carcass characteristics, immunity, and meat quality of broiler chickens. *Iran J Appl Anim Sci.* 2021;11(1):135-146.
 26. Underwood EJ, Suttle NF. The mineral nutrition of livestock. 3rd ed. Wallingford: CABI Publishing; 2001.
 27. Qu J, Zuo X, Xu Q, Li M, Zou L, Tao R, *et al.* Effect of two particle sizes of nano zinc oxide on growth performance, immune function, digestive tract morphology, and intestinal microbiota composition in broilers. *Animals.* 2023;13(9):1454.
 28. Surai PF. Antioxidant systems in poultry biology: superoxide dismutase. *J Anim Res Nutr.* 2016;1(18):1-17.
 29. Fathi M, Haydari A, Tanha T. Effects of zinc oxide nanoparticles on antioxidant status, serum enzymes activities, biochemical parameters and performance in broiler chickens. *J Livest Sci Technol.* 2016;4(2):7-13.
 30. Xie Z, Zhao Q, Wang H, Wen L, Li W, Zhang X, *et al.* Effects of antibacterial peptide combinations on growth performance, intestinal health, and immune function of broiler chickens. *Poult Sci.* 2020;99(12):6481-6492.
 31. El-Bahr SM, Shousha S, Albokhadaim I, Shehab A, Khattab W, Ahmed-Farid O, *et al.* Impact of dietary zinc oxide nanoparticles on selected serum biomarkers, lipid peroxidation and tissue gene expression of antioxidant enzymes and cytokines in Japanese quail. *BMC Vet Res.* 2020;16:1-12.
 32. O'Neill AM, Livant EJ, Ewald SJ. The chicken BF1 (classical MHC class I) gene shows evidence of selection for diversity in expression and in promoter and signal peptide regions. *Immunogenetics.* 2009;61(4):289-302.
 33. Li S, Lu L, Hao S, Wang Y, Zhang L, Liu S, *et al.* Dietary manganese modulates expression of the manganese-containing superoxide dismutase gene in chickens. *J Nutr.* 2011;141(2):189-194.
 34. Rimoldi S, Lasagna E, Sarti FM, Marelli SP, Cozzi MC, Bernardini G, *et al.* Expression profile of six stress-related genes and productive performances of fast and slow growing broiler strains reared under heat stress conditions. *Meta Gene.* 2015;6:17-25.
 35. Yuan D, Zhan XA, Wang YX. Effect of selenium sources on the expression of cellular glutathione peroxidase and cytoplasmic thioredoxin reductase in the liver and kidney of broiler breeders and their offspring. *Poult Sci.* 2012;91(4):936-942.